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THE PATHOLOGICAL CHANGES IN THE BONE MARROW IN AGRANULOCYTOSIS *

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One of the problems that confronts the hematologist of today is the question of the identity of agranulocytosis as a disease entity. The specificity of the condition has by some been vigorously denied, by others emphatically asserted, and by others again regarded as irrelevant or unimportant.

Evidence on this question may be garnered from both clinical and pathological view-points. This paper concerns itself with the latter.

In the medical literature of the pathology of agranulocytosis one finds almost complete chaos. This situation would appear to arise in part from an uncritical choice of cases studied, and in part from lack of appreciation of such variations in cellular composition as normally occur from one bone to another, even in the strictly normal individual. Thus Aubertin and Lévy [1] make no attempt to separate agranulocytosis pure and simple from pancytopenia, aplastic anemia and allied diseases, or from those leukopenic states obviously secondary to known toxic agents. Dodd and Wilkinson [2] describe the marrow of an 11 year old congenital syphilitic negro who died subsequent to arsphenamine therapy. Dameshek and Ingall [3] report 9 cases with pathological considerations drawn from 2. One had a progressive and eventually extreme anemia, the red blood cell count falling within a month from 4,000,000 per cmm. to 850,000 per cmm. The

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2nd case was a girl 4 years old with severe sepsis, bleeding from the mucous membranes, and a red blood cell count of 1,500,000 per cmm. Of the white blood cells 20 to 40 per cent were immature forms. Zadek [4] emphasized the presence of lymphocytes in the bone marrow and interprets the finding as indicating an active infiltration crowding out the myeloid cells. Yet, 2 of his cases were almost certainly leukemic in nature and showed progressive anemia and infiltration of the organs generally with large, immature lymphocytes, and a 3rd case showed a lymphocytosis of 11,000 per cmm. and lymphocytic nodules in the bone marrow. Such cases are instructive and serve to caution us against too great dogmatism regarding the pathological changes in leukopenic states. That they advance our knowledge of the pathology of agranulocytosis may be questioned.

On the other hand, Campbell and Murdock [5] describe the marrow of the tibia in their case (which incidentally was secondary to pneumonia) as fatty, without apparent recognition of the fact that the bone marrow from this region is normally fatty except in early childhood. Schultz, himself, has frequently been quoted as regarding the bone marrow in agranulocytosis as aplastic, yet Leon,[6] who reported on the histological changes in Schultz' cases, confines herself to the bare statement that neither adult polymorphonuclear neutrophils nor myelocytes were seen and that grossly the femur was partly red and partly fatty. Lichtenstein [7] in a most complete article reporting bone marrow studies in 18 of his own cases found a fairly uniform picture in the diaphysis of the femur, polymorphonuclear neutrophils absent or very rare, myelocytes usually entirely absent, myeloblasts present in small, occasionally moderate numbers, scattered lymphocytes, normal or increased numbers of megakaryocytes and plentiful red cells in all stages of development. Koch,[8] Kommerell,[9] and Baltzer [10] find in smaller groups of cases similar pictures, with the exception that plasma cells are also present in large numbers.

A further group of authors emphasize degenerative changes in the myeloid cells. Rotter [11] found many degenerated myeloblasts in the marrow of 1 of his cases. On this basis he has been quoted, without qualification, as finding degenerated myeloblasts in the marrow of agranulocytosis. This patient, however, clinically had a marked anemia and according to the author himself was questionably diagnosed agranulocytosis. Moreover, the marrow at autopsy was grossly invaded by bacteria, a fact which might well explain the observed

degeneration. Oppikofer[12] describes in 3 cases a few to many degenerated and degenerating myeloblasts. Photomicrographs accompanying his article show degenerated cells, the identification of which as myeloblasts is impossible from the photographs. Roberts and Kracke,[13] Hartwich,[14] Uffenorde,[15] and Pepper[16] concur in the observation of this degeneration in at least some of their cases. More recently Jaffé[17] has still further emphasized degeneration of the myeloid cells, especially the myelocytes in which he says the granules "swell and fuse with the cytoplasm."

The confusing influence of such varied reports is patent. Cases are described with the most diverse clinical and hematological pictures and the essential pathological bone marrow change of "agranulocytosis" has variously been described as aplasia, lymphoid infiltration and degeneration of existing cells.

It remained for Fitz-Hugh and Krumbhaar[18] to clarify the situation in their excellent study of agranulocytosis in 1932. They pointed out the virtually intact and unaltered state of the red cell series and, on the basis of finding a relatively cellular marrow containing, in so far as granules were concerned, only extremely early (stem) forms, they postulated for the true disease a maturation arrest of the white cells analogous to the erythroblast arrest in pernicious anemia. Their cases were for the most part typical and their pathological studies clear-cut and convincing.

These results were confirmed and amplified by the painstaking studies of Custer.[19] On the basis of 11 typical cases of agranulocytosis this author concluded that in the true disease the bone marrow showed a marked proliferation of myeloblasts, a failure of these cells to mature beyond that stage, a slight increase in the number of megakaryocytes and a moderate infiltration with lymphocytes and plasma cells. Custer concludes by saying that the "presence of a lesion of maturation specifically confined to the granulopoietic series, not reduplicated by diseases of known etiology, entitles idiopathic agranulocytosis, tentatively at least, to a place as a disease entity."

Some time prior to the publication of Custer's paper we had begun to collect and study all cases of agranulocytosis available to us on which we had sections of bone marrow. Only such were retained as appeared to be clinically typical and upon which we had adequate and properly prepared bone marrow sections. Typical agranulocytosis exhibits an acute onset with fever, prostration and ulcerative

stomatitis. The white blood cell count is almost invariably below 1500 per cmm. Few, if any, mature or immature granulocytes are found in the blood smear. The platelets are normal. The red blood cell count is normal or nearly so unless there be coexisting conditions producing anemia. There is no enlargement of lymph nodes not readily accounted for by adjacent sepsis, and the liver and spleen are not notably enlarged. There is little or no hemorrhagic tendency.

The group comprised 21 females and 4 males. They ranged from 24 to 85 years of age. Twenty-four died of the disease; 1 had a sternal bone marrow biopsy shortly before clinical and hematological improvement began. In all instances the leukopenia and neutropenia were extreme and unremitting. Minimum white blood cell counts varied from 120 to 1350 per cmm. with granulocytes comprising never more than 2 per cent of the total. Only 6 showed any appreciable anemia and in all this finding could adequately and logically be explained by preëxisting unrelated disease, such as chronic nephritis or advanced alcoholic cirrhosis of the liver. The lowest red blood cell count was 2,300,000 per cmm. in a patient (A–32–527) with clinical pernicious anemia which was rapidly improving under intensive liver extract therapy at the time of onset of the agranulocytosis. This case is of particular interest in view of Herndon's [20] report of a similar one in which, in spite of intensive liver therapy, typical agranulocytosis developed with a fatal outcome. The bone marrow, examined by Dr. William Bloom, showed no evidence of pernicious anemia but did show the characteristic changes of agranulocytosis, as described by Fitz-Hugh and Krumbhaar, and Custer. Herndon points out that such findings strongly militate against the view that liver extract may be effective in agranulocytosis and certainly evidence is afforded that the maturant factors of the red and white cell series are not identical.

None of the series showed any material reduction of platelets in the blood smear nor did any show a tendency to bleed from the mucous membranes or skin. In none could the diagnosis of aleukemic leukemia be properly entertained.

To bring out certain important similarities and differences in this series of fatal cases we have divided it into three groups on the basis of duration of symptoms before death.

The first group includes 7 cases where death occurred within 4 days of clinical onset. Typical of these is L. M. (A–32–487). This

55 year old woman, a known diabetic of some years standing, was seized rather suddenly 36 hours before entry with severe sore throat, marked dysphagia and vomiting. On admission she was found to be dehydrated and in mild acidosis, the latter feature being easily controlled by insulin and glucose. The faucial ring was injected and swollen and over the enlarged tonsils was a grayish slough. There was brawny infiltration of both sides of the neck. A few crackling râles were heard at the bases of the lungs. The red blood cell count was 5,100,000 per cmm. and the white blood cell count was 250 per cmm. In the blood smear no white blood cells other than normal lymphocytes could be found. The platelets appeared normal both in numbers and in form. The temperature was 103° F. The patient failed rapidly and died on the second day in the hospital. The bone marrow was typical of the rapidly fatal cases (Fig. 1). Red cell formation appeared to be normal. Megakaryocytes were slightly increased. No mature polymorphonuclear neutrophils or myelocytes were seen. The granulocytic elements were represented virtually entirely by stem cells (myeloblasts in Custer's terminology). Occasional scattered foci of lymphocytes occurred. Plasma cells were rare. Little phagocytosis was seen. Such cells as were present were not degenerated and mitoses were frequent among the stem cells, to which, however, further maturation appeared to have been denied.

In his rapidly fatal cases Custer found the predominating and indeed virtually the only granular cell to be the myeloblast. As a typical differential bone marrow count on such a case Custer found: myeloblasts 37.3; promyelocytes and myelocytes 2.8; red cell series 42.6; megakaryocytes 2.6; reticuloendothelial cells 9.1; lymphocytes 4.2; and plasma cells 1.3. His terminology and ours differ somewhat, but after personal communication it is obvious that we are in essential agreement. His myeloblast is our stem cell, his erythroblast our normoblast, and his normoblast our nucleated red cell. In comparison with the differential bone marrow count on a fulminating case we find on a similar case: stem cells 49; myeloblasts 7.2; lymphocytes 6; nucleated red cells, normoblasts and erythroblasts 29.4; reticular cells 1.6; and megakaryocytes 6.6. Thus, it is apparent that in those cases where death took place within 4 days of the clinical onset the essential lesion consists in an increase of the very early granular cells (stem cells) and a lack of further maturation.

For this latter phenomenon we propose the term granulocytic anakmesis.*

The second or intermediary group including 11 patients dying from 5 to 10 days after clinical onset gives a somewhat less uniform picture in the bone marrow. Two of them, dying 7 and 10 days respectively after onset, had essentially the same picture as was seen in the earlier group.

Of the remaining 9, which may be regarded as illustrative of the intermediary group, patient F. G. (A–32–466) is typical. She was a 59 year old female who entered the hospital with a 4 day history of fever, chills, sore throat and cough. Four months previously she had been treated in the hospital for hypertension and marked chronic nephritis. Four days before the second entry she was taken ill suddenly with cough, chills, vomiting and sore throat. On entry the tonsils and pharynx were greatly injected. In the left lung were signs of bronchopneumonia. The temperature was 100° F., pulse 95, respirations 25. The urine showed a large trace of albumin and the blood non-protein nitrogen was 70 mg. per cent. The red blood cell count was 2,700,000 per cmm. with the hemoglobin 53 per cent. The white blood cell count was 900 to 1000 per cmm. on repeated determinations during her stay in the hospital. No white blood cells other than mature lymphocytes were ever seen in the blood smear. The clinical course was rapidly downhill. On the third day the pharynx showed extensive necrotic ulceration and the temperature had risen to 104° F. The throat culture showed no diphtheria bacilli or hemolytic streptococci. She died on the 8th day after the onset of disease.

Autopsy revealed a variety of pathological features: chronic glomerular nephritis, cardiac hypertrophy, old adhesive pericarditis and pleuritis, bronchopneumonia, pulmonary edema and congestion, and necrotic pharyngitis. The spleen weighed 350 gm. and showed microscopically an increase in fibrous tissue. The vertebral bone marrow, the only specimen obtained, was grossly dark red. Microscopically the cellularity was about normal, the red cell series present in normal proportions; megakaryocytes were very numerous. The myeloid series was represented by scattered stem cells and a very rare myelocyte. Polymorphonuclear neutrophils were entirely

* This word was suggested by Dr. John H. Finley, Jr., of Harvard University and we should like to acknowledge our indebtedness to him.

absent. Plasma cells and lymphocytes were numerous, the latter usually in small clumps.

The bone marrow of the remaining 8 cases of the intermediary group could be described in the same words as this typical case — with one exception. The number of lymphocytes and plasma cells varied widely. By and large, however, in comparison with the early cases the proportion of stem cells had greatly decreased while that of the plasma cells and lymphocytes had correspondingly increased. Megakaryocytes and red cells were as before, essentially normal. Whenever sections from the femur were available, they showed a picture identical to that in the vertebra and sternum. The fruitless proliferation of stem cells had spread to the normally acellular regions and appeared to have burned itself out in a vain effort to supply to the peripheral blood normal cells of the granular series.

The third, or late, group included 6 cases where death took place after an illness of more than 10 days. A. A. (A–33–661) was typical. Six months before entry she had had a sore throat for a week, not requiring medical attention. The present illness began 6 weeks before entry with a mild sore throat which became much worse 3 weeks later. During the 2 weeks prior to admission she had had chills, high fever, headache and swollen lymph nodes in the neck, together with extreme dysphagia. On examination the tonsils were large, swollen and covered with yellow exudate. Bean-sized and tender lymph nodes were palpable in the neck. The urine contained a very slight trace of albumin. The white blood cells numbered 1000 per cmm. on entry and fell gradually to 500 per cmm. At no time were any polymorphonuclear neutrophils seen, or any early granulocytes, such as might suggest the presence of leukemia. Platelets in the smear were always very numerous. Her course was steadily downhill. The temperature was rather constantly maintained at 102° F. Pentnucleotide (N. N. R.) was given intramuscularly in 50 cc. daily doses. On the 8th day in the hospital she died, some 3 weeks after the onset of the acute symptoms.

Autopsy showed many ulcers of tonsils, pharynx and the entire gastrointestinal tract, early bronchopneumonia, congestion and edema of lungs, liver and brain. Grossly the bone marrow from the vertebra and sternum was dark red, that from midfemur and humerus pale yellow. The vertebral sections showed an occasional stem cell, in all others they were very rare (Fig. 2). Mature granulocytes

were absent, megakaryocytes numerous. The red cell series appeared undisturbed. Most striking in all marrows were the myriads of plasma cells. In addition numerous large phagocytic cells containing red cells and nuclei were seen. Custer finds in his "marked cases" of agranulocytosis rather more myeloblasts (stem cells), more promyelocytes and myelocytes, less erythroblasts (normoblasts), and far more lymphocytes than in his "early cases." But it should be pointed out that by "marked cases" he refers to those suffering from the disease for many months. It is debatable whether these should be classed with the more acute forms. In our "late cases" — that is, in individuals dying over 10 days after the onset — we have uniformly found that the stem cells have largely given way to plasma cells, which cells now formed the predominant white cell. The red cell series still remains essentially unaltered. A typical differential count is that on JP–83a, dying 3 weeks after clinical onset: stem cells 5.2; myeloblasts 1.8; lymphocytes 19.4; plasma cells 37.4; nucleated red cells, normoblasts and erythroblasts 23.6; reticular cells 4.6; megakaryocytes 5; unclassified 3.

The remaining 5 cases of this group showed essentially identical pictures in their important features; marked myeloid hypoplasia, many plasma and lymphoid cells, essentially normal red cells, and many megakaryocytes. Only the phagocytosis was an inconstant feature, as it was marked in half the cases of this group and in a quarter of the intermediary group.

Our 1 remaining case, H. C. (S–33–3544), may best be classified as in the early recovery phase at the time of sternal biopsy. During the previous year she had had two attacks of severe sore throat without actual ulcerations. On these occasions the white blood cell count was about 2000 per cmm. with 0 to 5 per cent polymorphonuclear neutrophils. Both times she received pentnucleotide and recovered. At no time was there any anemia. The present complaint was severe sore throat for 2 days. Her throat on entry was markedly injected and she was acutely ill with a high fever. Otherwise the physical examination was normal. The white blood cell count was 1500 per cmm. with no polymorphonuclear neutrophils. That day a biopsy of the sternal marrow was taken. The next day the white blood cell count was 4450 per cmm. with 44 per cent granulocytes, mostly young polymorphonuclear neutrophils. Concomitantly she improved clinically and in 1 week she was entirely well with a white

blood cell count of 9300 per cmm. with 70 per cent polymorphonuclear neutrophils. It is interesting, in view of recent evidence that pyramidon may be a cause of agranulocytosis, that this patient took that drug regularly before and after each attack. In spite of this regular use of the drug the white blood cell count taken periodically has been entirely normal in the past 16 months.

Microscopic study of the sternal marrow showed a picture of rapid regeneration (Fig. 3). The tissue was crowded with all stages of myeloid cells from early myelocytes to young polymorphonuclear neutrophils. Only an occasional lymphocyte and plasma cell were to be found. As might be expected, the red cell series and megakaryocytes appeared normal. The intense activity gave the impression that some stimulus had recently been given to or some block removed from myeloid development. Custer has pointed out that neither the neutropenia of overwhelming sepsis nor that of arsphenamine poisoning is accompanied by a bone marrow change similar to that described in agranulocytosis. In sepsis he found 26.6 per cent segmented polymorphonuclear neutrophils and a total of 61.8 per cent cells of the myelocyte stage or older. Similarly, we found in a case of overwhelming sepsis with a white blood cell count of 1400 per cmm. (A–35–328) 9.2 per cent segmented forms and 64.2 per cent granular cells of the myelocyte stage or older. The bone marrow picture of overwhelming sepsis is not that of agranulocytosis.

From an analysis of the 25 typical cases of agranulocytosis it would appear, as Fitz-Hugh and Krumbhaar first suggested, that in the rapidly fatal cases the bone marrow shows stem cell hyperplasia and myeloid anakmesis without notable changes in the red cell series and that as the survival of the patient becomes longer the stem cells gradually and somewhat irregularly give way to plasma cells and lymphocytes. It may be hypothesized that early in the disease there is a compensatory increase of the number of normally occurring stem cells (myeloblasts) in a vain effort to overcome the maturation arrest and that in the latter stages these stem cells disappear and a coincident increase of lymphocytes and plasma cells occurs.

Summary and Conclusions

1. The uniformity of the pathological changes in the bone marrow suggests that agranulocytosis is probably a disease entity.

2. Rapidly fatal cases show lack of maturation in the granular series and hyperplasia of the stem cells.

3. Cases where death took place after a longer period usually show hypoplasia of myeloid tissue with the coincident appearance of many plasma cells and lymphocytes.

4. In no case were there obvious changes in the red blood cell series, or any degenerative changes in the white blood cell series.

5. The recovery stage is characterized by rapid development of the stem cells into myelocytes, metamyelocytes and polymorphonuclear neutrophils — a sequence of events which would appear to substantiate Fitz-Hugh and Krumbhaar's and Custer's contention of a maturation arrest.

6. For this maturation arrest we suggest the term granulocytic anakmesis.

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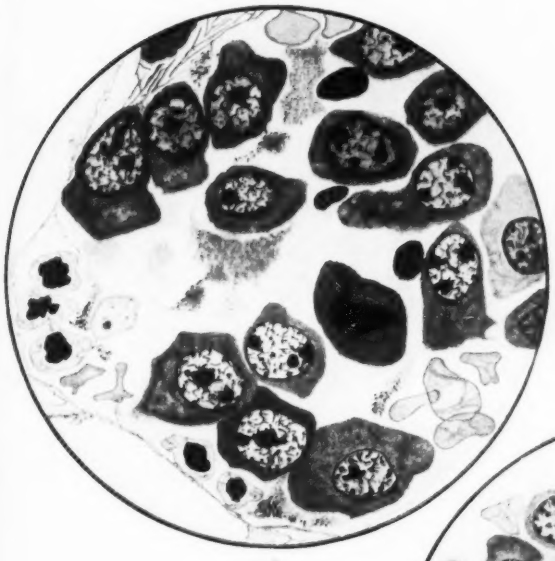
DESCRIPTION OF PLATE

PLATE 1

FIG. 1. Bone marrow from a rapidly fatal case of agranulocytosis. Virtually all of the white cells are stem cells, some showing active mitosis. (A–32–13.) × 1000.

FIG. 2. Bone marrow from a case of agranulocytosis where death took place 3 weeks after clinical onset. Stem cells are virtually entirely replaced by plasma cells and lymphocytes. One phagocytic cell engulfing red cell. (J P–83a.) × 1000.

FIG. 3. Bone marrow from a case of agranulocytosis just prior to improvement in the peripheral blood picture. Rapid development of myelocytes with the presence of an occasional young polymorphonuclear neutrophil. (J P–74.) × 1000.



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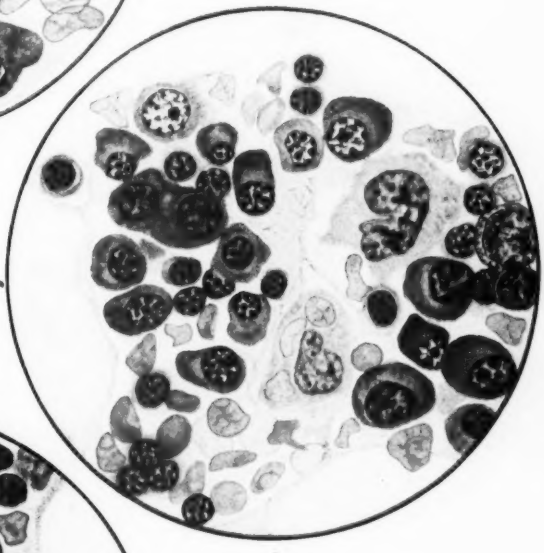

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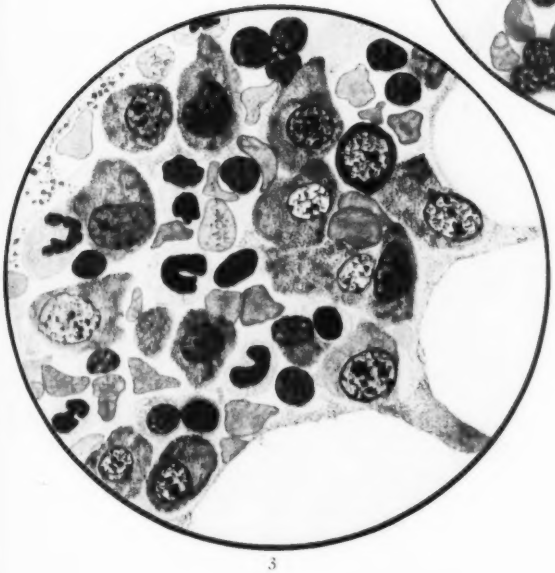

3

A SPECTROGRAPHIC STUDY OF LEPROUS LESIONS *

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These determinations were made with the idea that significant modifications might occur in Na, Ca, Mg, P, Fe and, perhaps, other elements in the lesions as compared with unaltered tissue of the same sort. It was thought that if a marked chemical change paralleled the development of distinctive lesions, something might be done to re-establish normal ratios between the elements that would be of therapeutic value. In this paper we wish to report a beginning in this direction made possible by the fact that histopathological studies on leprosy are being carried on in the same laboratory in which Dr. Gordon H. Scott and his associates are using the technique of histospectrography.[1-4] We are very grateful to Dr. Scott for his help and advice. We have made a spectrographic examination of leper lesions from 6 cases, the examination involving comparison with five "normal" skins taken to represent roughly normal conditions, and with three other such skins on which chemical analyses were run. All eight of the controls afford opportunity for semiquantitative estimates (of the "greater than" or "less than" variety as described by Scott and Williams [2]) of P/Ca, Na/Ca, Mg/Ca and Fe/Ca ratios in leper lesions as compared with normal skin; and we have worked out numerical values for the P/Ca and Na/Ca ratios based upon the spectrographic findings on three chemically analyzed normal skins.

Material

The leper tissues were collected at biopsy from five lepers at the United States Marine Hospital, Carville, Louisiana, with the permission and cooperation of Dr. O. E. Denney and through the kindness of Major S. Simmons, at autopsy by Dr. E. DeCoursey of the Board of Health Laboratory, Ancon, Canal Zone. The former are referred to as L 11 to L 15. The lesion in each was divided into five pieces which were qualitatively alike as far as could be determined

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macroscopically. These were immediately fixed (1) in 10 per cent formalin in absolute alcohol, (2) 10 per cent formalin, (3) Regaud's fluid (3 per cent potassium bichromate 4 parts, and formalin 1 part), (4) formalin-Zenker's for histological examination and (5) frozen with dry ice and kept frozen until studied spectrographically in St. Louis. The latter, designated L 3 and L S 3, were fixed in the formalin-alcohol mixture and mailed to St. Louis therein. On arrival equivalent parts were used for histological and spectroscopic examination. It is necessary to specify the source and the histopathology of the particular lesions studied spectroscopically because others of different nature may have a different mineral content.

L 11. Patient P. A. 1049, Mexican, male, 42 years of age, mixed leprosy with cutaneous type predominating, 4 years duration with no treatment. *Biopsy:* removal of the ear lobules.

Epidermis: Slight hyperkeratosis; 35–75 μ thick, average 45 μ. Considerable pigment. No leukocytic invasion. No epithelial pegs.

Papillary Layer: Almost obliterated owing to absence of pegs and flattening of dermal papillae. The remains of the layer constitute the subepithelial marginal band of Unna.[5] The thickness of this band varies from 36–40 μ. It is made up of collagenic fibers with a few fibroblasts between them. The elastic fibers, normally present in the papillary layer, are much reduced in number and no traces remain of the sense organs of the dermal papillae in any of these leprous specimens. The band has a blood supply greatly reduced from that of the original papillary layer, but it is separated from the underlying reticular layer by a zone of moderately distended vessels. The relative immunity of this subepithelial marginal band to penetration by the leproma cells, emphasized by Unna, holds for this and other specimens in our series.

Reticular Layer: Much thickened by a leprous nodule, which is only partly divided into lobules by septa of connective tissue (seldom more than 75 μ wide), blood vessels (about normal in size), many sebaceous glands and hair follicles. The latter are slightly atrophic. The nodules are made up chiefly of histiocytes, foam cells, lymphocytes, fibroblasts, polymorphonuclear leukocytes, tissue mast cells and tissue eosinophiles, cited in order of frequency. The term "plasma cell" is used in the sense of von Marschalkó,[6] which is different from that of Unna, who employed it as synonymous with "protoplasmic cells," which clearly include histiocytes (macrophages, monocytes, epithelioid cells, and so on) as well. No giant cells like those illustrated by Wade's [7] Figure 12 were seen in this or in specimens from any of the other cases. Bacilli are numerous in the histiocytes as typical "cigar packs," or intracellular globi. Globi of the same sort are found in a few fibroblasts and occasionally in vascular endothelial cells. Bacilli, not clumped as globi, are occasional in the outer cells of sebaceous glands, duct cells of sweat glands and in the tissue fluid. Much larger, roughly globular masses of bacilli, which are not intracellular but intercellular and have been called "giant globi" by Unna, are rare. Denney has commented upon them in

a recent paper.[8] We hope to present in another communication our views as to their nature, relation to the small intracellular globi, and the whole very important question of lymphatic involvement. Here we are concerned only with their occurrence and size in an attempt to grade the tissues. In L 11 they were not numerous and the largest had a maximum diameter of 15 μ.

Only a small amount of *subcutis* is included in the sections. Contrasted with other specimens, nodule formation is the least advanced, giant globi least frequent and the normal structure of the skin least disturbed. Conversely, L 11 exhibits more fatty areolar tissue, well formed sebaceous glands, and in the nodules a higher percentage of tissue mast cells and plasma cells than any of the others.

L 12. Patient W. M. 619, white, male, 30 years of age, mixed leprosy with cutaneous type predominating, 10 years duration under routine chaulmoogra oil treatment. *Biopsy:* removal of nodule on forearm.

Epidermis: Atrophic, tendency to hyperkeratosis; 30–135 μ thick, average 95 μ. Little or no pigment. Slight leukocytic invasion. Epithelial cells swollen, increase in size approximately 33 per cent. No epithelial pegs.

Papillary Layer: Ironed out owing to lack of pegs and dermal papillae. The subepithelial marginal band is 10–150 μ wide, much less dense than in L 11 and limited internally by a zone of dilated vessels.

Reticular Layer: The leprous nodule is of fairly uniform consistence, since it is not broken up by sebaceous glands, hair follicles, fatty areolar tissue or large bands of connective tissue; for all these are absent. The sweat glands are atrophic but the blood vessels are not noticeably changed. The nodule is more cellular and less fibrous than in L 11. The connective tissue increases gradually as the subcutis is approached and becomes disposed in strata and whorls. In the nodule, intercellular spaces are marked, particularly near the external margin, suggestive of much tissue fluid, more indeed than in any of the other specimens. The most abundant cells in the nodules are histiocytes. There are many foam cells, a few lymphocytes and occasional polymorphonuclears. Plasma cells are rare and no tissue mast cells or tissue eosinophiles are seen. Bacilli are abundant as small intracellular globi in histiocytes and free in the tissue. Giant globi are fairly numerous. The walls of the lymphatics, which contain them, show more marked hyperplasia than in any of the other specimens except L 3, so that when cut at an angle they simulate giant cells. No true polykaryocytes are seen. The *subcutis* is not included in section.

L 13. Patient F. H. 971, Mexican, male, 36 years of age, with marked cutaneous leprosy of 5 years duration. Has had no treatment. *Biopsy:* removal of nodule on forearm.

Epidermis: 20–80 μ thick, average 35 μ. In one area, about 1 mm. wide, it is distinctly atrophic being approximately 20 μ thick. The cells are largest and vacuolated where the epithelium is thickest. Much pigment. No leukocytic invasion. No epithelial pegs.

Papillary Layer: Almost flattened out by absence of epithelial pegs and great reduction in size of dermal papillae, which only penetrate epidermis to depth of 20 to 50 μ and are not numerous. The subepithelial marginal band is about half the thickness of the overlying epidermis. It is mostly collagenic, but in its outer lamina rather more elastic fibers are demonstrable by resorcin fuchsin than in the other cases. A few pigment cells are present in the band.

Reticular Layer: Thickened, by growth of leprous nodule which is not much separated into lobules by connective tissue or epithelial derivatives. No sebaceous glands or hair follicles. On the average there is only one duct of sweat gland per section, the lumen of which is almost closed. In the nodule, histiocytes are most numerous, plasma and foam cells are rare, the blood vessels are open with normal walls. No tissue mast cells or eosinophiles were seen but long search was not made. Bacilli are quite granular, but very numerous especially as small globi intracellular in histiocytes. There are many giant intercellular globi. The largest was 50 μ in diameter (measured in 10 per cent formalin-fixed material). Only one instance of proliferation of lymphatic endothelium leading to pseudo-giant cell formation was noted.

Subcutis: Only partly included in section. No fatty areolar tissue anywhere in specimen.

L 14. Patient F. V. 514, white, male, 35 years of age, mixed type of leprosy with cutaneous lesions predominating, 19 years duration, routine treatment with chaulmoogra oil. *Biopsy:* removal of nodule from nape of neck.

Epidermis: Atrophic and smooth; 25–40 μ thick, average 30 μ. Small amount of pigment. No leukocytic invasion or pegs.

Papillary Layer: This is practically absent, because the pegs and dermal papillae are lacking. The subepithelial marginal band is narrower and less pronounced than in any other specimens of the series. It varies in width from 10–25 μ. In some cases the cells of the nodule come into direct contact with the inner surface of epithelium. But in that portion fixed in alcohol-formalin the band is wider and more like tissue from the other cases.

Reticular Layer: Enlarged by nodule formation. Not broken up into lobules by hair follicles, sebaceous glands or sweat glands; for these are absent, except in the alcohol-formalin-fixed piece in which there are two small sebaceous cysts. It consists of histiocytes, foam cells in moderate numbers, a few lymphocytes, plasma cells, and occasional polymorphonuclears together with fibroblasts and connective tissue fibers disposed in thin bands. There are no tissue mast cells or eosinophiles. Bacilli are very numerous, free and in small globi. Giant globi are more frequent in the Regaud-fixed fragment than in specimens from any of the other cases, but in other pieces from this case they are not unusually abundant. No *subcutis* is available. No fatty areolar tissue or giant cells are seen.

L 15. Patient J. P. 1050, Mexican, male, 30 years of age, mixed type of leprosy with cutaneous lesions markedly predominant, 8 years duration with no treatment. *Biopsy:* removal of nodules from face.

Epidermis: Atrophic, 30–55 μ thick, average 40 μ. It contains considerable pigment. Leukocytic invasion is very rare and there are no pegs.

Papillary Layer: Reduced by absence of pegs and dermal papillae. The subepithelial marginal band is 30–100 μ thick and chiefly collagenic. The most dilated venules in the series are just internal to the band but the number of vessels is not increased.

Reticular Layer: Greatly increased in thickness by nodule formation. The nodule is of fairly uniform consistence, being very little broken up by sebaceous glands and hair follicles which are distinctly rare. Connective tissue bands are but feebly developed and there is no fatty areolar tissue except in the fragment fixed in formalin-Zenker in which the included lesion is less severe. The nodule in all specimens of L 15 is made up of histiocytes, with plasma cells more numerous than in any of the other cases except L 11, lymphocytes, foam cells and a few tissue mast cells; but only one multinucleated giant cell much vacuolated and about 35 μ in maximum diameter was seen. Bacilli are numerous, free, in small globi and in giant globi. The latter, though not particularly frequent, are the largest seen in the series of leprosy specimens. One was oval in shape in section with maximum diameter of 125 μ and minimum of 108 μ. In general, tissue from this case is rather like that of L 11.

L 3. Autopsy No. 10563, Board of Health Laboratory, Ancon, Canal Zone.

Epidermis: 20–80 μ thick, average about 50 μ. Heavy pigmentation. No leukocytic invasion. Inner surface is very uneven owing to extension of numerous short (20–120 μ, average 70 μ), pointed epithelial pegs — a feature more marked in this than in any of the other specimens.

Papillary Layer: Atrophied as compared with normal, but not ironed out, like many of the others, because dermal papillae alternate with the pegs. It contains more than the usual number of pigment-holding cells. The subepithelial marginal band is indistinct. Increase in collagenic fibers is only moderate and decrease in elastic fibers is limited to approximately the inner half of the reticularis. Moreover, the thickness of the band is variable (30–100 μ, average 70 μ) depending on the proximity of the nodules to the epithelium.

Reticular Layer: Contains layer of relatively small flattened nodules, each having a length (parallel to epidermis) of about 150–800 μ and maximum thicknesses of 40–300 μ. Between them stretch bands of connective tissue, blood vessels, a few nerve fibers and the ducts of sweat glands. The nodules are backed internally by a feltwork of thick strands of connective tissue containing many elastic fibers. In the feltwork are nodules of the same and larger size which extend toward the subcutis as far as the section goes, namely 3–4 mm., and are accompanied by fatty aveolar tissue in amount only slightly less than normal. The composition of the nodules differs. Those of the outermost layer are made up for the most part of foam cells and histiocytes, the former being most numerous. Lymphocytes and plasma cells are scarce. Only two tissue mast cells and no eosinophiles were seen. In the deeper nodules there are with the foam cells, a fair number of lymphocytes and plasma cells, some fat, many giant globi attaining a maximum diameter of 50 μ, and numerous giant cells whose maximum diameter is about 70 μ and whose largest number of nuclei in a 6 μ section is 10 per cell. In some cases, the giant cells are merely sections through hyperplastic

walls of lymphatics in which cell boundaries are lost. Bacilli in both superficial and deep nodules are granular and fragmented.

L S 3 is from the same autopsy as L 3, selected because on gross inspection it showed so few signs of leprous change that the results of spectrographic examination would be significant as compared with L 3.

Epidermis: Similar.
Papillary Layer: Similar but slightly more elastic tissue remaining and tissue mast cells distinctly more numerous.
Reticular Layer: Outer layer of small nodules similar. Deeper nodules less than half as large. The cytology of the nodules is similar, except that there are fewer giant globi and giant cells than in L 3.

The normal tissues consisted of pieces of skin removed from the upper left chest of five white cadavers, age about 60 years or more. These are known as N 1 to N 5 inclusive. In addition, skin from three individuals was analyzed chemically for P, Na and Ca by Miss C. C. Buhrmester, as well as studied spectrographically. The specimens were from the abdomen of a male and female cadaver of about the same age as N 1 to N 5 and from the chest of a male, aged 53, who died of empyema (our Department of Pathology Autopsy No. 6184), obtained through the courtesy of Dr. H. L. McCordock. These are called M, F and A, respectively. We are grateful to Dr. R. J. Terry for permission to collect skin from the cadavers.

Spectrographic Analyses

(*A*) *Handling of Material*

The method of obtaining the spectra of the materials has been fully described elsewhere.[1, 2, 3] It consists essentially of burning 2–4 cmm. (per spectrum of 15″ exposure) of tissue in a high frequency spark and photographing the emission spectrum with a small quartz spectrograph. The material to be burned was first cleansed of subcutaneous fat by scraping with a knife, then cut in strips about 2 mm. square, and up to a centimeter or two in length, in the case of normal skin. With the leprous lesions, however, the strips were necessarily somewhat shorter. They were held in small pyrex glass tubes for introduction into the spark. The portion of each leper lesion selected was always immediately adjacent to those examined histologically. With each of the normal skin cases M, F and A, the

strips for the spectrographic record were taken at three different places from the rather large area of skin (25 to 100 sq. cm.) used for the chemical analysis. The whole area of skin was cleansed of subcutaneous fat before the strips were cut.

The spectra, in general, show spectral lines characteristic of Ca, K, Na, Cu, Mg, Fe, P, Si, C and occasionally Ag. These lines are visible on the accompanying plates, which exhibit representative portions of the sets of spectra obtained for the materials employed. Beside the spectra are listed the designations employed in the description of material. Only the lines actually measured up are indicated — for the others, see previous papers.[1,2,3] Also very evident are the continuous bands due to the passage of the spark through the air.

The density ($= \log_{10}$ opacity) of one line each for P, Na, Ca, Mg and Fe was measured with an electrical densitometer [4] in each spectrum examined. The results were then averaged for each kind of tissue. For instance, the four spectra available for leper Case 11 yielded four values for the P line density, and the average of these gave the P line density characteristic of the case. The results of such treatment of all the densitometer readings are given in Table I, which indicates also the number of spectra measured for each material. All spectra were taken with the same exposure time (15 seconds) and on the same type of plate (Eastman 50).

(*B*) *General Quantitative Estimates*

The working hypothesis is: If in the spectra of two similar tissues A and B, the Mg lines are of about the same density, while the P lines of A are much more dense than those of B, it can be concluded that the P/Mg ratio is greater for A than for B. Any other two elements can be taken, besides Mg and P. It is not sufficient to fix attention on the lines of only one element, since errors would ensue from the considerable variations in the rate of consumption of material by the spark. It is assumed that the spectra to be compared are taken on the same type of plate and with the same exposure time, and given as nearly as possible the same development. The spectra here considered do not very well satisfy the development requirement, since they were taken over an interval of some months without particular reference to the scheme of presentation now employed. However,

the relations to be pointed out are sufficiently pronounced not to be invalidated by this criticism.

The hypothesis requires that with a given general type of tissue (for example, skin, which burns brightly, as distinct from fat, which melts and sputters in the spark) the line intensity increases with increasing amounts of the element in the tissue. With the low metallic element concentrations encountered in nearly all tissues, and with the large amount of organic material present which tends to "ballast" spark phenomena against alteration due to changes in metallic content, self-reversal and quenching of the spectral lines of the elements of interest should be slight; consequently, the requirement should be satisfied.

The outstanding difference between leper and normal tissue seems, for the cases at hand, to concern calcium and phosphorus. From Table I the average Ca line density for leper Cases 11–15 is 0.65, while for the eight normals it is 0.76; but the P relations are in the other direction — leper 0.67, normal 0.44. Hence we conclude the P/Ca ratio is larger for leper lesions than for normal skin. This can be seen in comparing Figures 1 and 2 for relative P and Ca line strengths. Almost the same numerical relations exist for the Ca and Fe line densities, and hence the same conclusion follows for the Fe/Ca ratios, see again Figures 1 and 2. While inspection of the Ca and Mg densities indicates a tendency for the Mg/Ca ratio to be greater for the leper material, the differences are perhaps not large enough to establish anything in view of the large variations observed from sample to sample of the same type of material, and in view of possible errors arising from the fact that the spectra concerned were on various plates. Likewise, the Na/Ca ratios show only a tendency to be greater in the leper material.

Leper Case 3 is of special interest. Here we have spectra of two lesions of the same type but of different severity from the same patient on the same plate. Further, both samples were taken at autopsy. Thus the comparison is free from certain of the qualifications which apply to those already mentioned. In the first place it is very evident, not only from Table I, but also from Figure 1, that the P/Ca ratio is greater for the heavier lesion (L 3) than for the lighter one (L S 3). The Mg/Ca ratio shows again the tendency in that direction, as does the Na/Ca ratio. But the Fe/Ca ratio seems to be the same in both.

This last point, perhaps, accounts for the high Fe/Ca ratio in leper Cases 11–15 as compared with the normals; the former samples were taken at biopsy, the latter generally months after death, while both samples for Case 3 were taken at autopsy at the same time. Since the spectrum of whole blood (Scott and Williams,[2] Figure 4, Spectrum 1) has been found to be extremely rich in Fe from the hemoglobin, it is probable that in spite of the low vascularity of leper lesions enough blood was retained after biopsy to account for the

TABLE I

Mean Line Densities for Spectra of Leper and Normal Tissues

Case	Number spectra	Line densities				
		Ca	Mg	P	Fe	Na
Leper L 11	4	0.73	0.51	0.62	0.76	0.3
L 12	4	0.54	0.51	0.66	0.51	0.82
L 13	4	0.70	0.55	0.66	0.82	0.91
L 14	3	0.58	0.51	0.66	0.66	0.82
L 15	3	0.69	0.64	0.77	0.66	0.89
Average		0.65	0.54	0.67	0.68	0.85
Normal N_1	4	0.73	0.53	0.38	0.34	0.78
N_2	4	0.79	0.46	0.36	0.41	0.74
N_3	4	0.63	0.44	0.40	0.32	0.84
N_4	4	0.67	0.34	0.27	0.31	0.70
N_5	4	0.87	0.48	0.41	0.38	0.72
M	12	0.86	0.44	0.72	0.71	1.04
F	12	0.93	0.56	0.67	0.73	1.07
A	10	0.68	0.37	0.36	0.38	0.88
Average		0.76	0.45	0.44	0.45	0.85
Leper L 3	10	0.96	0.83	0.87	0.76	0.92
L S 3	10	0.94	0.73	0.58	0.73	0.75

strong Fe lines in the leper spectra, particularly as the samples were immediately frozen in solid CO_2 rather than preserved in a liquid fixative. It should be remembered, also, that the normals were taken from the chest or abdomen in cadavers which had been hanging vertically for some time, and signs of extensive blood drainage into the lower portions of the bodies were evident.

Finally it is instructive to apply the above method of interpretation, for the P/Ca and Na/Ca ratios, to the normal skin cases M, F and A, for which these ratios were determined chemically (Table II, columns 5 and 7). Referring again to Table I, Ca (M) < Ca (F),

while P (M) > P (F); hence, P/Ca for M is greater than for F, which checks the chemical results. The comparison of A with either M or F is not quite of the same sort, and must be handled differently. We may note that the difference of the P and Ca line densities for A, (0.36 − 0.68 = −0.32) is less algebraically than the corresponding difference for either M or F (− 0.14 and − 0.26). Since the smaller the amount of P present in proportion to the Ca, the smaller should be this difference, we again check the chemical results. A similar treatment holds for the Na/Ca relation. The differences in Na and Ca line densities for M, F and A are respectively 0.18, 0.14 and 0.20 which are in the same sequence as the chemical findings 14.1, 10.5 and 18.1 for the Na/Ca ratios.

(*C*) *Numerical Estimates*

In the foregoing we used the difference in the P and Ca line densities for a particular kind of tissue as an index for the P/Ca ratio in that tissue, and similarly for Na/Ca. The qualitative validity of this choice of index was shown by the agreement obtained for the normals M, F and A with the chemical findings. We shall now correlate the indices for M, F and A numerically with the chemical results, and by graphical methods find the P/Ca and Na/Ca ratios for the other materials concerned.

As previously remarked, the spectrographic plates employed differed somewhat in development, and possibly also in regard to the intensity (more specifically, number of sparks per second; the nature of each was probably constant) of the analyzer spark during the period in which they were taken. It was desirable to correct for these influences as much as possible before making numerical estimates. From consideration of the probable spark phenomena, and of the behavior of the characteristic (density-versus-intensity) curves for the photographic plates with varying degrees of development, it seemed plausible to fix upon the density of some particular portion of the airband system, averaged for all the spectra on a plate, as a means of such correction. An airband density was found, then, for each plate. The mean for all the plates concerned was determined. This mean value divided by the value for a particular plate gave a factor by which all line densities for that plate were multiplied. These factors were applied to give the corrected, and somewhat ab-

breviated, set of line densities shown in Table II, columns 1, 2 and 3.

The factors ranged from 0.77 for the plate with the M and F spectra to 1.31 for the N 1 to N 5 plate. No great claims are made for the precise accuracy of these corrections, but it is hardly to be doubted that they are in the right direction: they work proportionate increases in line densities for the fainter plates and proportionate

TABLE II

*Mean Corrected Line Densities for Spectra of Leper and Normal Tissues, and Computed Element Ratios. The Ratios Marked * were Determined Chemically*

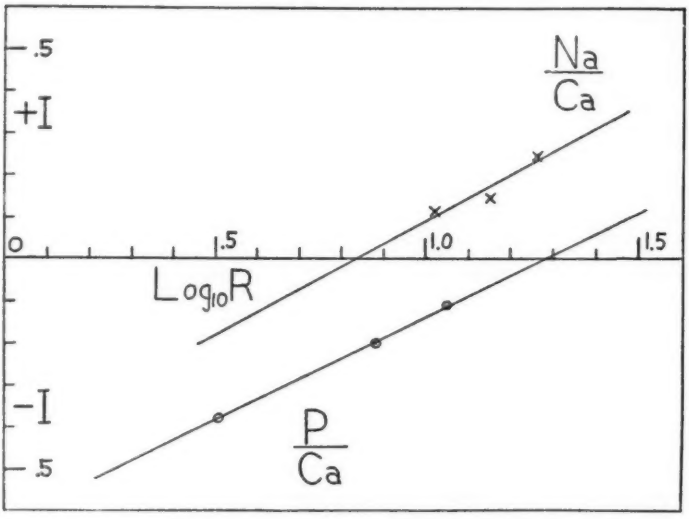
Case	Corrected line densities			P/Ca		Na/Ca	
	P	Na	Ca	I_1	R_1	I_2	R_2
Leper L 11	0.66	0.88	0.76	−0.10	12.00	0.12	11.50
L 12	0.69	0.86	0.59	0.10	30.90	0.27	21.40
L 13	0.70	0.94	0.74	−0.04	15.80	0.20	16.20
L 14	0.68	0.85	0.60	0.08	28.20	0.25	20.00
L 15	0.79	0.91	0.72	0.07	26.90	0.19	15.50
Average					22.70		16.90
Normal average N_1–N_5	0.47	0.99	0.96	−0.49	1.90	0.03	7.95
Normal M	0.55	0.80	0.66	−0.11	*11.30	0.14	14.10*
F	0.52	0.83	0.72	−0.20	* 7.55	0.11	10.50*
A	0.44	1.06	0.82	−0.38	* 3.25	0.24	18.10*
Average					3.95		10.30
Leper L 3	0.85	0.90	0.94	−0.09	12.60	−0.04	5.90
L S 3	0.57	0.73	0.92	−0.35	3.70	−0.19	3.00
Column	1	2	3	4	5	6	7

decreases for the blacker ones. Furthermore, the general quantitative estimates are unaltered by the corrections.

Column 4, Table II, gives the differences between the P and Ca corrected line densities for the various materials, which are taken as indices I_1 for the P/Ca ratios R_1 of the materials. For three of these materials (M, F and A) we know R_1 from chemical measurements. Now, to a rough approximation, spectral line density is proportional to the logarithm of the intensity of the light producing the line; and, for minute quantities of an element, the intensity should be proportional to the amount of the element entering the spark. Thus, to get

a curve for purposes of computation, I_1 is plotted against $\log_{10} R_1$ in Graph 1 for M, F and A, the points being shown as circles in the graph. A straight line is drawn as nearly through the three points as possible. Then the R_1 values for the other materials are secured by fitting their I_1 values to this straight line. For instance, I_1 for leper Case 11 is -0.10; the corresponding $\log_{10} R_1$ value is given by the graph as 1.08, and the antilog of this is $12.0 = R_1 = P/Ca$ for Case 11. The other cases are handled in the same way.

The indices I_2 for Na/Ca are given in column 6, Table II; and their $R_2 = \frac{Na}{Ca}$ values are computed as above from the upper Na/Ca line in Graph 1, and are given in column 7, Table II. The crosses are the points from cases M, F and A, which establish the line.



GRAPH 1. Differences (I) in corrected line densities in terms of element ratios (R).

For obvious graphical reasons an R value is likely to be most accurately determined when the degree of extrapolation is least, *i.e.* when its I values fall within the range covered by those of cases M, F and A. The corresponding R ranges for these 3 cases are about 3–12 for P/Ca, and 10–19 for Na/Ca; so, in general, more re-

liance should be placed on computed R values within, or close to, these limits than on values far outside them. However, the P/Ca calibration points are sufficiently separated to permit plausible extrapolation for, perhaps, all of the R_1 values given.

Discussion

Herman Brown [9] has determined, among other things, P, Na and Ca for a series of normal human skins by chemical means. The samples were taken at autopsy, freed from subcutaneous fat by scraping and cover an age range from the fetal stage to 82 years. The location is not given. There are other figures on Na and Ca by the same author in an earlier paper [10] for skin "from the ventral region between the clavicles and the symphysis pubis." The ratio P/Ca from his figures ranges from 2.6 to 5.5 for the age group 60 to 80 years; our Table II gives 3.95 for the mean of the eight normals. For Na/Ca in the same age group Brown's figures range from 9.2 to 11.7; Table II gives 10.3 for the normal average. The P/Ca (but not the Na/Ca) for L S 3 also agrees with these chemical results.

These agreements with previously published findings fortify our P/Ca and Na/Ca results for leper Cases L 11 through L 15. The mean P/Ca for the 5 cases is 22.7; Brown's normal results for the age group (30 to 42 years) concerned range from 4.6 to 6.8 and average about 6.0. We may also note that $22.7/6.0 = 3.8$ happens to be comparable with $12.6/3.7 = 3.4$ for L 3 and L S 3. Thus it seems safe to conclude that the P/Ca ratio for the cases at hand is around three times higher in well developed leper lesions than in normal skin. As to Na/Ca, the average 16.9 for the leper lesions is within the range for the corresponding age group as found by Brown (12.0 to 20.0, average around 14.5). It should be noted that the small difference in Na/Ca ratios mentioned in (*B*), and further shown in column 7, Table II, was real enough for the samples at hand; it simply becomes of no consequence when the large variation of Na/Ca with age, as established by Brown's figures, is taken into account.

Consideration of the location of the leprous lesions brings in, however, a factor which we have been unable to check. In the five biopsies they were removed from the ear lobule, forearm, forehead, nape of the neck, and face. These are all exposed parts of the body in contrast with the areas ordinarily covered with clothes, from which we

removed our supposedly normal skin samples. We have not been able to find any data in the literature as to the presence or absence of a difference in the P/Ca ratio of exposed and unexposed parts of the body under normal conditions. Our own attempts to secure samples of normal skin at autopsy from the same exposed areas for spectrographic analyses have not been successful because of the mutilation involved in collecting them. But we think it very unlikely that the high P/Ca ratio in our leprosy cases is due primarily to a regional difference in chemical composition of the skin.

The available evidence points to the conclusion that the deviation from the normal of the ratio is related to the length of time which elapsed since the leprous condition was first diagnosed clinically. Reference to Table II, column 5, shows that this ratio is high in L 12, 14 and 15 in which the disease had been established for 10, 19 and 8 years and lower in L 11 and 13 of 4 and 5 years duration. But this may not mean so much because there is no assurance that the particular lesions studied were the first detected. In other words, those with unusually high P/Ca ratios may have developed comparatively recently in persons in which the disease had already been well established elsewhere. Brown has found considerable variation in ratio values for undiseased skins from cases of approximately the same age. However, an accidental correlation due to natural variability is improbable where 5 cases are concerned even though the correlation does not involve, for instance, a direct proportionality between ratio and duration.

A high P/Ca ratio signifies either an increase in P relative to Ca, or a decrease in Ca relative to P, or both. We accordingly attempted to discover whether a correlation exists between a high P/Ca ratio and a high percentage in volume of fatty aveolar tissue, sebaceous glands and true leprous nodule consisting of cells charged with bacilli or with products of their degeneration (foam cells) as compared with other components making up most of the remaining bulk of the skin, namely: epidermis, hair follicles, sweat glands, connective tissue, blood vessels and tissue fluid all grouped together.

Obviously the calculations leave much to be desired from the point of view of accuracy for they were based entirely on the impressions gained by repeated microscopic comparisons of the tissues. Another consideration must be mentioned that may detract from their value. The fifth piece of tissue, into which each specimen removed at bi-

opsy was divided, was frozen and again subdivided, part being used for spectrographic analysis and part for further histological control. The last named was not so well preserved for histological examinations as the four others intentionally fixed for this purpose and, since in L 14 and 15 there was a qualitative difference in the pieces, as already described, it is possible that the tissue burned was not always absolutely comparable to those on which our histopathological account is based.

However this may be, L 12 with highest P/Ca ratio (30.9) showed no fatty areolar tissue; whereas L 11, with lowest P/Ca ratio (12.0), had more than L 15; while L 13 and 14 possessed no demonstrable fatty areolar tissue. Lack of correlation with volume of sebaceous glands was likewise evident. No sebaceous glands were found in tissue from L 12. These structures were most marked in L 11. But the relative volume of leprous cells was noticeably greater in L 12 with the highest ratio than in L 11 with the smallest one. Moreover, in L 11 the bacilli were less numerous and giant globi less conspicuous than in other members of the series. The other cases (L 13 and 14) were difficult to grade and showed no definite correlation of the same sort. Our findings in tissues of L 3, which had to be kept separate from the others because they alone of the leprous tissues were removed at autopsy, suggest, however, the same correlation because the ratio was higher in L 3 with large deep nodules than in L S 3 with smaller ones.

Unfortunately there are no data in the literature on the actual richness in P of leprous nodules of small size dissected free of surrounding tissue. It is merely an assumption that they contain much P and that our correlation between large total volume of cells containing bacilli and their products and high P/Ca ratio partly explains the height of the ratio. We have examined frozen and alcohol-formalin-fixed specimens from the 5 cases by the method of micro-incineration, which is not useful for the demonstration of P, but reveals many mineral constituents including Ca, and have observed that the mineral residue left by the leprous cells is not very extensive. In blood serum Wooley and Ross [11] report, on the basis of chemical analyses, total amounts of Ca and inorganic P which average, for 47 cases of leprosy, well within normal limits as represented by 15 controls. The diffusible Ca, however, in 53 cases averaged considerably lower than in the 15 controls. In a later paper [12] these au-

thors mention the probability that "diffusible calcium" and "available calcium" are essentially synonymous, and advance the opinion that nerve, muscular and bone changes in leprosy may be in part due to this effective Ca deficiency. Perhaps Ca starvation of tissue may be aggravated in the actual foci of leprous infection and may contribute to the abnormally high P/Ca ratios that we have observed.

The absence in our results of a pronounced difference between leper and normal tissue in regard to Mg/Ca ratio is not surprising in view of the considerable chemical similarity of Ca and Mg. That is, a disease condition tending, say, to reduce Ca content might for this reason have an effect at least in the same direction on Mg. As to the Fe/Ca ratios, it might at first sight seem logically unsound to discount the high Fe/Ca ratio in leper tissue as compared with the normals simply on account of the difference in the way samples were taken and because of the behavior of one leper case (L 3).

Summary and Conclusions

1. The P/Ca ratios in 5 leper cases studied are on the average probably three times those in normal skins from the same age group. The Na/Ca, Mg/Ca and Fe/Ca ratios show no notable variations from the normal.

2. A fair correlation is obtained in the 5 leper cases for P/Ca ratio with known duration of disease and volume of leprous cells in tissue analyzed spectrographically. It may be conditioned by increase in P, decrease in Ca, but probably by both.

3. The method of histospectrography, as developed by Scott and his collaborators, can evidently be used for the study of small pieces of tissue removed at biopsy which would be altogether insufficient in amount to permit of routine chemical analysis by ordinary methods. Once the spectrograms have been taken, essentially the same procedure is employed for the determination of ratios between several elements; whereas the chemical estimation of each element would be different and in some cases very involved.

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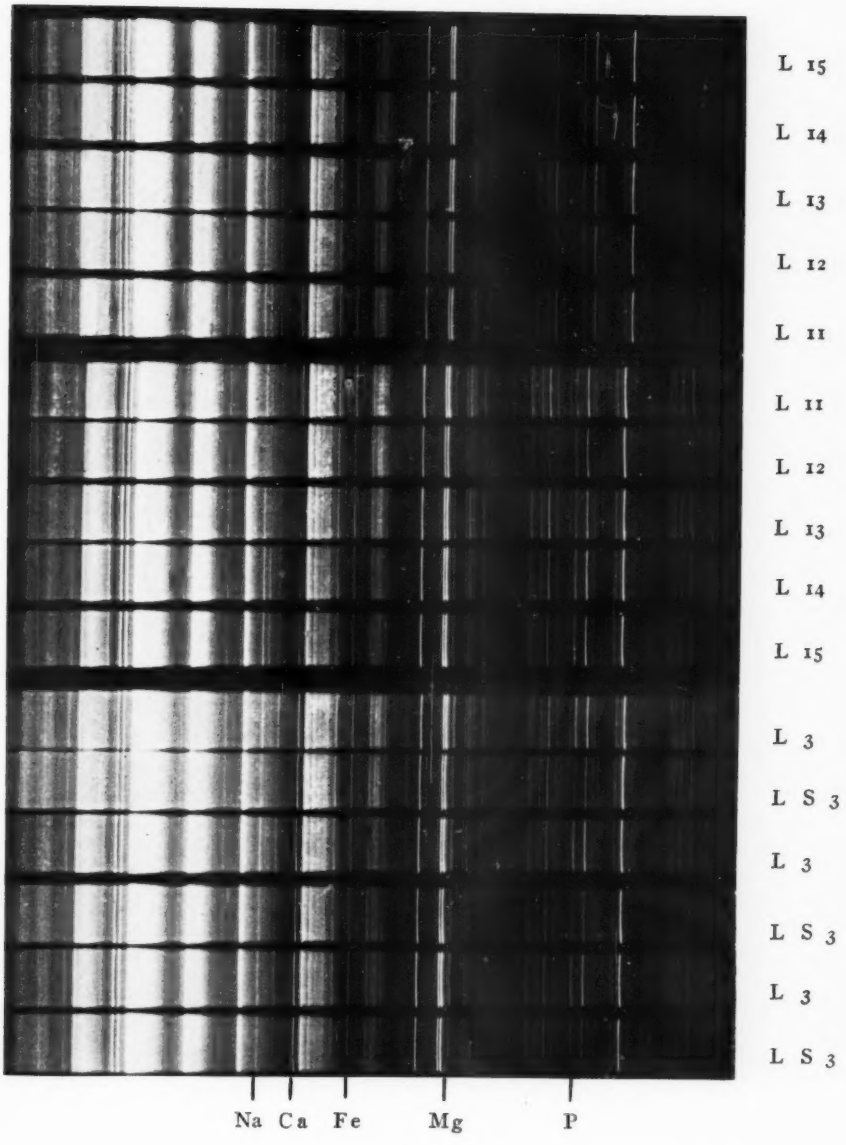
DESCRIPTION OF PLATES

PLATE 2

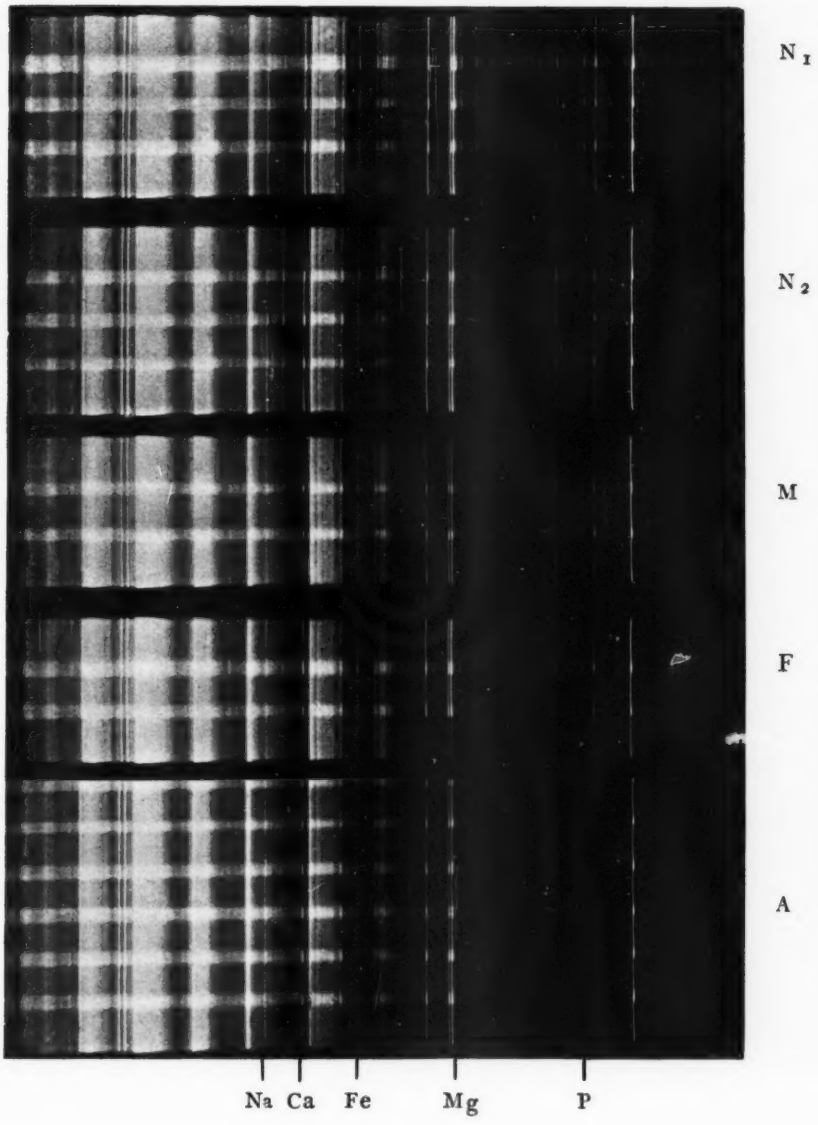
FIG. 1. Representative spectra (enlarged) of leprous tissues. The numbers of the cases are indicated in the margin and the lines measured for the particular elements studied are identified below. The two groups of spectra shown for L 11 to L 15 are reproduced from different plates. The spectra from Case 3 (L = heavy lesion and L S = light lesion) are from still another plate. The particular Mg line indicated is visible only with difficulty in these prints. It is immediately to the right of the two very bright lines.


L 15
L 14
L 13
L 12
L 11
L 11
L 12
L 13
L 14
L 15
L 3
L S 3
L 3
L S 3
L 3
L S 3
Na
Ca
Fe
Mg
P

1

PLATE 3

FIG. 2. Representative spectra of normal tissues for visual comparison with the leprous ones illustrated in Figure 1. Case and line designations are arranged as in Figure 1. N 1 and N 2 are from one plate, M and F from another and A from a third. Note that the phosphorus lines, relative to the calcium lines, are weaker than in Figure 1. This is in accordance with the conclusion reached in the text that the Ca/P ratio is less in leper lesions than in normal tissue.



2

LESIONS IN THE AURICULOVENTRICULAR CONDUCTION SYSTEM OCCURRING IN RHEUMATIC FEVER *

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In spite of the fact that disturbances in conduction and rhythm in the heart form an almost invariable and conspicuous finding in active rheumatic fever, remarkably few histological studies have been reported on the tissue of the conduction system in this disease. Thus, for example, the microscopic findings in this tissue from cases of conduction disturbances in rheumatic fever are reported only in single instances each in the excellent monographs of Mönckeberg [1] and Mahaim.[2] In addition to these two authors, scattered reports on very limited material have been made by Gerhardt,[3] Bramwell,[4] Löw,[5] and Naish and Kennedy.[6] The reported findings refer to infiltrations of the bundle with inflammatory cells (in one instance with giant cells), swelling of collagen, "fibrous degeneration of the auriculo-ventricular bundle of His with the presence of a calcareous nodule almost obliterating the bundle," and, more significantly, "lymphocytic infiltration in the region of the node and trunks."

Because of the very limited nature of these reports it is not surprising that various explanations of the cause of the conduction disturbances have been advanced, such as myocardial fatigue, toxic injury, the presence of specific lesions and accumulation of fluid in the synovial sac which was assumed to surround the conduction system in man.

Published reports by one of us (L. G.) with collaborators [7-12] have indicated a high incidence of inflammatory and vascular changes occurring in various parts of the heart in rheumatic fever. It seemed of interest, therefore, to study systematically the conduction system in a representatively large series of cases of unquestionable rheumatic nature in which other affections which might implicate this tissue could be reasonably ruled out.

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Material and Methods

The findings in 110 hearts form the basis of this report. Of these, 60 presented active rheumatic fever as manifested by the presence of fibrinous pericarditis, acute verrucous endocarditis, Aschoff bodies, eosinophilic swelling of the collagen and other inflammatory changes of the myocardium. Twenty-five cases represented inactive rheumatic material according to the specifications laid down by Rothschild, Kugel and Gross.[13] These cases showed no Aschoff bodies in the myocardium. The remaining 25 cases formed a non-rheumatic control series representing various age periods from birth to the ninth decade of life. This series was carefully studied to eliminate past or present hypertension, subacute bacterial endocarditis and syphilis. A careful study of the clinical records and pathological specimens was made in order to correlate, if possible, the findings with the clinical course of the disease. Electrocardiographic records were studied, when available, with the same object in mind.

The technical methods used were essentially those previously described by Gross and Ehrlich.[7] We have found that a carefully differentiated hematoxylin and eosin stain, as well as a combination of Weigert's elastica and Van Gieson's connective tissue stains, is quite satisfactory for the study of the pathology of the conduction system. The lighter staining of the neuromuscular apparatus, together with its richer vascularity, heavier elastic and collagenous framework and peculiar architectural and topographical relations, render the identification of the specific tissue relatively simple. A variety of stains considered more selective for histological studies of this region is listed by Todd.[14]

Since the studies herein reported are based on an examination of the routine tricuspid valve section obtained by the use of the standardized method of Gross, Antopol and Sacks,[15] the description of the pathological material will concern itself only with the auriculoventricular nodal tissue and the bundle of His as far as and at times including the division into the ventricular limbs. For the sake of brevity this horizontal auriculoventricular conduction system will be referred to as "the bundle." No attempt will be made in this report to describe the findings in the auricular and ventricular ramifications of the Purkinje system since these appear to be considerably less important in the mechanism concerned with the usual conduction disturbances occurring in rheumatic fever.

Topography of the Horizontal Auriculoventricular Conduction System in the Human Heart

Studies on the histology and topography of the conduction system in the normal human heart have been far more extensive and complete than those on rheumatic hearts. Excellent descriptions are available from the works of His,[16] Tawara,[17] Mönckeberg,[1] De Witt,[18] Tandler,[19] Van der Stricht and Todd,[20] Clerc,[21] Géraudel,[22] Mahaim,[2] Taussig [23] and Todd.[14] For purposes of clarity it seems advisable to present a short description of the Tawara node and the bundle of His * up to its divisions into the ventricular limbs. This description, which is based on our own observations, will emphasize certain points which will be pertinent to our discussions of the findings in rheumatic fever.

The actual site of the horizontal auriculoventricular conduction system (node of Tawara and bundle of His) with respect to the surrounding tissues shows a moderate amount of variation. There are, however, certain general rules concerning this site and particularly concerning the modification in the histology of the specific tissue as one proceeds from the posterior origin of the system toward its anterior termination into the left and right ventricular limbs. The nodal tissue, or posterior end of the horizontal conduction apparatus, starts in the neighborhood of the coronary sinus ostium as a somewhat loose structure which is either applied very closely to the subendocardial tissue of the right auricle or is covered by a wedge of auricular myocardium of varying thickness (Fig. 1). At this point the base of the tricuspid valve generally lies approximately 6–7 mm. below the crest of the septum in the adult heart, and the conduction system on cross-section slopes toward the right from above downward.

The cells themselves may be closely intermingled with the auricular myocardial fibers and merge imperceptibly with them. They are thin, generally running parallel with the auricular fibers in this region, but they may have a whorled architecture. At this site either the bundle itself or the collagenous extension of the septum fibrosum immediately adjacent to the bundle tissue generally contains large

* Todd does not believe that the auriculoventricular node exists as a special formation. Certainly the structure of the specialized tissue which makes up the so-called Tawara node merges imperceptibly with the more anterior portions of the auriculoventricular conduction system (bundle of His).

blood vessels. Indeed, it is the presence of these large blood vessels within the bundle or in its immediate environs that aids in the identification of the tissue as being nodal or representing the first portion of the bundle of His. Occasionally, at this site, fat cells are intermingled with the nodal tissue. These are sometimes also distributed throughout the auricular myocardial wedge and, contrary to the view held by Engel,[24] apparently do not necessarily represent signs of damage.

In this posterior portion of the horizontal conduction system the left border of the bundle lies against the dense collagenous extension of the septum fibrosum and the outlines of the bundle are extremely irregular. Approximately 4 mm. anterior to this site the bundle is generally more compact, tending to take a roughly triangular form on cross-section with the apex of the triangle cephalad, with one angle sloping somewhat lower toward the right ventricle and the third angle at a higher level sloping toward the left ventricle. The outlines of this portion of the bundle as a whole are generally more clearly defined although still somewhat irregular. The bundle continues to occupy a position to the right of the collagenous septum fibrosum extension; it is somewhat higher placed, with respect to the crest of the interventricular septum, and almost invariably has a large portion of the right auricular myocardial wedge clothing its right face. The left face of the bundle lies against the dense collagenous extension of the septum fibrosum. Below the oblique border of the bundle triangle, and separating it from the crest of the interventricular septum, there is generally a strand of collagenous tissue of varying thickness. The cells of the bundle are generally narrow or medium sized and may either run obliquely or be arranged in whorls. At this site large and medium sized vessels are still frequently found and, not infrequently, fat cells may be irregularly dispersed throughout the bundle or be more aggregated on its right oblique face, thus forming an irregular barrier between the bundle and the auricular myocardial wedge.

Several millimeters anterior to this site (a point at which the base of the tricuspid leaflet is approximately level with the upper border of the septal crest) the bundle tends to take a roughly cylindrical or oval shape on cross-section (Fig. 2). It now occupies approximately the middle of the septum fibrosum, consists of generally large cells, fairly compact, running anteroposteriorly for the most part, and ar-

ranged in basket or bundle form. The whorled appearance has now disappeared. In place of large vessels there are often seen arterioles. Fat cells are infrequent and the collagenous band of the septum fibrosum surrounds the bundle on all its aspects.

At a variable distance anterior to this site (3–5 mm.) the bundle begins to fork into its right ventricular and left ventricular limbs. On cross-section the bundle is generally represented by a somewhat flattened triangle. The cells are large, again beginning to take on an oblique or longitudinal direction parallel with the crest of the interventricular septum and with its walls, the basket arrangement of the fibers may or may not be present, the cells are fairly compact, and dense collagenous tissue surrounds this flattened triangle with its beginning forking of the limbs. If one traces these forked limbs toward the right and left ventricles either at this site or somewhat anteriorly to it, one sees a rapid transformation of the cells into the very large, pale Purkinje fibers. Fat cells are infrequently found in the bundle at this site and the vessels are practically all capillaries.

As a general rule it may be stated that the concentration of elastic and collagenous framework is generally greater in the more posterior aspects of the bundle, chiefly at the nodal site, and becomes increasingly sparse as one progresses anteriorly. The elastic tissue, when present, tends to be concentrated around the blood vessels. The more anterior aspects of the bundle can also be determined by the fact that the right auricular wedge has completely disappeared and the septum fibrosum consists of a strong band of collagenous tissue clothed by endocardium on both sides. The human horizontal conduction apparatus apparently is very poorly supplied by lymphatics. These do not appear to have any predilection site with respect to the location of the section. Vascular channels with prominent endothelial cells are often seen in various portions of the bundle. These may represent collapsed lymphatics or veins.

Histological Findings in Non-Rheumatic Control Cases

As previously stated, the histological studies herein reported are based on an examination of the routine tricuspid valve section (T. V.) obtained by the use of the standardized method of Gross, Antopol and Sacks. This section generally includes that part of the horizontal auriculoventricular conduction system which lies just be-

hind the cylindrical portion of the bundle of His. At times, however, the section was taken either anterior or posterior to this site. In making comparisons of the bundle tissue in the various cases the above described differences in topography, vascularity and histology of these sites were borne in mind.

In order to establish a base line for comparison it is necessary to state briefly the findings in the bundles of 25 non-rheumatic hearts which were obtained at autopsy from patients dying of a variety of diseases other than those which implicate the endocardium. Many of these diseases were of an infectious nature (generally bronchopneumonia). Some were associated with a bacteremia. In the sections studied no appreciable abnormalities were noted either in the distribution or structure of the specific cells of the horizontal conduction system. As was previously indicated, the interstitial framework between the cells of the specific tissue varies somewhat with the site from which the tissue was obtained. None of the non-rheumatic control cases showed a conspicuous increase in this fibro-elastic framework. Only 1 case (a child, 9 years old, dying of a *Staphylococcus aureus* bacteremia following osteomyelitis) showed edema of the bundle and a very definite increase in the interstitial elastic fibers. Apropos of the latter, it may be stated that elastic fibers are either very delicate or non-existent until the latter part of the third or the beginning of the fourth decade of life, after which they become increasingly conspicuous, particularly in the posterior portions of the horizontal auriculoventricular conduction system (nodal portion). Elastic fibers, when present, tend to be concentrated around blood vessels. The collagenous framework between the cells of the bundle is extremely delicate and becomes somewhat more plentiful only from the fifth decade of life on.

During the first four decades of life a very sparse scattering of lymphocytes, occasional macrophages, plasma cells and other mononuclear cells may be seen between the cells of the bundle. Whether these occur in strictly normal material it is difficult at present to state. In making comparisons with the rheumatic material these cells of inflammatory origin will be considered as increased only when they are definitely more numerous than those noted in this control material.

With regard to the histology of the blood vessels in the bundle, as observed in control material, it may be stated that intimal elastifica-

tion is seen in approximately half the cases from the fourth decade on. The capillaries were found dilated in approximately one-third of the cases and dilated lymphatics were seen in approximately one-sixth of the cases.

In view of the findings to be presented in the rheumatic material, it is of considerable interest to note that the collagenous extension of the septum fibrosum abutting against the bundle showed a few blood vessels in 6 of the 25 non-rheumatic control cases. Scattered lymphocytes were exceedingly rare at this site. Elastification of the septum was inconspicuous in this control material.

Histological Findings in Cases of Rheumatic Fever

Included in the 60 cases of active rheumatic fever were patients who died during what appeared to be the first attack of rheumatic fever, those in which there were repeated attacks previous to the fatal outcome, and patients with chronic valvular disease dying of heart failure whose hearts showed macroscopic and microscopic evidence of activity. All of the 60 cases showed Aschoff bodies in the myocardium. Only 2 of the 60 showed Aschoff bodies in the bundle tissue (Fig. 3). These were both from patients dying during a first attack. Evidently, therefore, this rather rare presence of Aschoff bodies is not sufficient to account for the frequent conduction disturbances during life.

Of greater frequency was the accumulation of inflammatory cells in the bundle (Figs. 4, 5 and 6). This occurred in 13 cases (22 per cent) to an extent greater than that met with in the control material. The preponderating cell was generally the lymphocyte. Occasionally, polymorphonuclear leukocytes were the more conspicuous of the cellular elements. Together with these there were noted plasma cells, macrophages and, at times, young fibroblasts, although these occurred in much smaller proportions. The cells were generally irregularly distributed throughout the bundle tissue, but tended to be more concentrated at the borders of the bundle as it abuts against the adjacent tissue.

Edema of the bundle, which is represented by the appearance of sometimes lightly basophilic material lying in the interstices between the cells, was found in 9 cases (15 per cent). This was found only in the patients dying during the first attack (Figs. 3 and 4). As regards the supporting framework of the bundle, there did not appear to be

any increase in collagen in the cases falling into this group. On the other hand, the elastic tissue framework appeared to be definitely increased in 22 cases (37 per cent). This was noted even after making due allowance for variations in the site of the bundle, as well as for age periods. In the majority of instances this increase in elastic tissue was found in the cases of chronic valvular disease where the individual died in decompensation but still showed active rheumatic disease (Aschoff bodies, and so on) histologically.

Considered as a whole, exudative phenomena were found in some portions of the bundle during the active phases of rheumatic fever in over one-third of the cases. Serial sections would undoubtedly have shown a higher incidence. It may be stated that the greatest increase in the incidence of inflammatory phenomena, as well as in their extent, was noted in the clinically very active cases. As is well known, conduction disturbances are much more likely to take place in these very active cases.

A very striking finding in the cases of active rheumatic fever was the presence of a variety of vascular lesions. These lesions were qualitatively similar to a number of those described as occurring in the myocardial coronary tree in active cases of rheumatic fever.[10] Thus, 15 of the 60 cases (25 per cent) showed intimal musculo-elastic hyperplastic changes (Fig. 5). As mentioned in previous reports, this lesion is highly characteristic, if indeed not specific, of rheumatic fever. Twenty cases (33 per cent) showed intimal proliferation with minimal or no elastic changes. Two cases showed thrombi lying in small veins. In 1 case the smooth muscle nuclei showed vacuolization. Three cases showed marked medial hypertrophy. Many cases showed dilated capillaries and some showed thickened ones.

Considered as a whole, these vascular changes occurred much more conspicuously and frequently than the exudative phenomena. To this must be added the fact that not all the cases studied were represented by a section through the posterior portion of the horizontal auriculoventricular conduction system which contains the larger vessels. Had such sections been available for study, it appears likely that approximately twice the number of cases indicated above would have shown these vascular lesions. This would bring the incidence of vascular lesions in general in the active rheumatic cases to approximately 66 per cent, and the incidence of intimal musculo-elastic hyperplastic lesions to about 50 per cent of the cases.

One of the most interesting findings in the active rheumatic material, and one which possibly plays a rôle in the production of conduction disturbances, is the frequent occurrence of inflammatory disturbances in the collagenous extension of the septum fibrosum which abuts against the bundle tissue (Figs. 4 and 6). As previously indicated, this collagenous tissue shows one or two vessels in the proximity of the bundle in approximately 24 per cent of the control non-rheumatic cases. A very rare scattering of lymphocytes is occasionally found near these vessels. In contrast to this, a conspicuous increase in vascularity, as well as in inflammatory cells, was found in 29 cases (48 per cent) of the active rheumatic series. These findings were noted chiefly in the cases where death took place during a first attack, especially in the clinically active cases. The inflammatory cells were similar to those described as occurring in the bundle. In 2 cases the inflammation of the septum was so extraordinarily marked that the collagenous band was converted into a mass of active granulation tissue (Fig. 6). In 2 cases the blood vessels showed intimal musculo-elastic hyperplastic lesions. In 1 case Aschoff bodies were present in the septum. In a significant number of these cases the elastic tissue of the collagenous extension of the septum fibrosum was definitely increased.

The findings in the 25 cases of inactive rheumatic fever, where most of the individuals died of extracardiac causes, are conspicuous by their paucity. These were in all respects similar to those described as occurring in the non-rheumatic control series. Evidently, therefore, the cases of rheumatic fever which are of so mild a nature as to permit of complete clinical and anatomical inactivation of the inflammatory process, leave no appreciable clinical or histological evidence of disturbance in the horizontal auriculoventricular conduction system.

Discussion

Electrocardiographic tracings were available for study in only 16 of the 60 active rheumatic fever cases. From this limited material it was impossible to establish a direct relation between the extent of the abnormal electrocardiographic findings and the intensity of the inflammatory lesions in the bundle and in the tissue surrounding this structure. In spite of the fact that usually only one or two sections were studied from each case, at least 66 per cent of the cases showed

exudative or vascular lesions within the bundle or within the tissue in its immediate neighborhood. As indicated above, it seems probable that serial sections would have raised the incidence of these lesions to an even higher level.

The finding of the frequent involvement of the collagenous extension of the septum fibrosum appears to be of considerable interest both from the functional point of view as well as from considerations concerning the spread of the infection. Heart block due to contiguity spread of presumptively syphilitic lesions from the root of the aorta to involve the bundle of His and septum fibrosum has been observed by Sohval.[25] The very high incidence of vascular and exudative lesions in the septum fibrosum extension in cases of active rheumatic fever suggests the possibility that these may be due to a contiguity process from the root of the aorta, the ring of the aortic cusps (chiefly the posterior), as well as from the auricular myocardial wedge. This would account for the frequency with which the bundle of His is involved in rheumatic fever and for the functional evidences of this involvement as indicated by the frequent conduction disturbances found in this condition. An added factor in the mechanism of the production of these conduction disturbances may lie in the topographical relations of the bundle. Inasmuch as one part of the conduction system is entirely surrounded by the relatively rigid collagenous tissue, this non-yielding mantle probably limits swelling and expansion of the bundle tissue when this is involved in an exudative process. As a consequence, compression of the cells takes place. There does not appear to be any evidence to support the assumption that edematous fluid within lymphatics may be responsible for these functional changes. Furthermore, as is well known, the horizontal conduction system in the human heart is not surrounded by a lymphatic sheath such as is seen in animals.

The absence of inflammatory and vascular lesions in the group of cases which showed chronic valvular disease without the presence of activity in the myocardium suggests the possibility that many of these processes, if indeed not all of them, may be capable of healing without leaving appreciable characteristic residua. That this is possible in the case of some vascular lesions in rheumatic fever has already been indicated elsewhere.[10] Surprisingly enough, this may also hold true for elastification of the bundle and the collagenous extension of the septum fibrosum, inasmuch as elastification of these sites

is not infrequently encountered in the active cases but is seldom seen in the inactive ones. As indicated before, an alternative hypothesis would be that the chronic valvular disease cases without activity represent a clinical course which was extremely mild even though continued over some period of time, and that eventually complete healing took place. Perhaps under such circumstances involvement of the bundle is minimal, so that one does not have to assume restitution to integrity of advanced lesions.

Finally, it may be stated that in the course of active rheumatic fever there occurs a series of exudative and vascular phenomena which are sufficiently frequent and intense to account for the high incidence of conduction disturbances.

Summary and Conclusions

One hundred and ten human hearts have been examined in order to determine the nature and frequency of the lesions occurring in the Tawara node and bundle of His in rheumatic fever. Sixty of these cases represent active rheumatic fever, 25 cases inactive rheumatic fever, and 25 cases non-rheumatic material. It has been shown that in active rheumatic fever there occurs a variety of inflammatory and vascular phenomena within the horizontal conduction system as well as in the surrounding tissue. Even when studied in few representative specimens from each bundle, the incidence of these lesions was approximately 66 per cent in the active material. It is probable that a study of more sections would have indicated a higher incidence. Very few of these lesions are of a specific or highly characteristic nature. The inactive rheumatic cases showed few pathological changes. This is in keeping with the functional differences observed as between these two groups. Attention has been called to the high incidence of inflammatory lesions in the collagenous extension of the septum fibrosum and a discussion of the possible mechanisms concerned with the spread of the rheumatic infection to the bundle tissue is given. A description of the topographical relations of the horizontal conduction system in the human heart, together with the findings in 25 non-rheumatic control cases is also given.

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DESCRIPTION OF PLATES

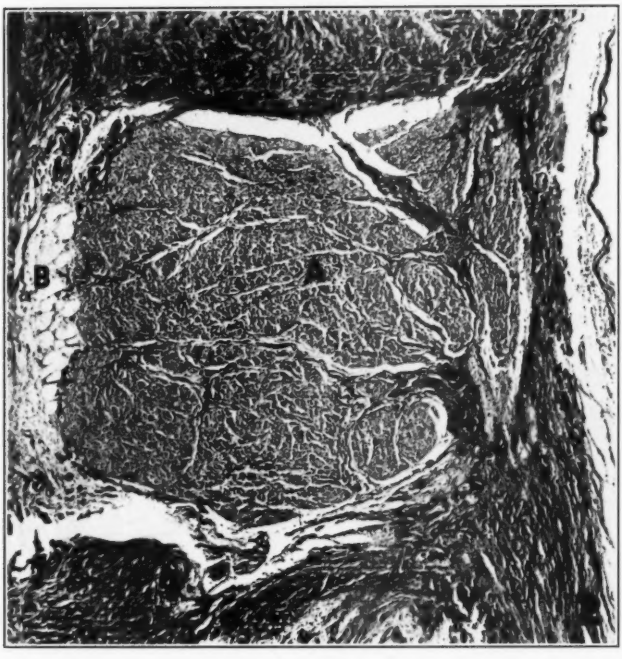
PLATE 4

FIG. 1. Cross-section of horizontal conduction system from non-rheumatic control case taken from its posterior portion. Age 31 years. Weigert's elastic and Van Gieson's connective tissue stain. Low power.

A = bundle tissue. Note whorled arrangement of the cells; B = artery of the bundle; C = sparse auricular myocardial fibers lying on right side of bundle; D = collagenous extension of septum fibrosum lying on left side of bundle.

FIG. 2. Cross-section of horizontal conduction system from non-rheumatic control case taken from its middle portion. Age 62 years. Weigert's elastic and Van Gieson's connective tissue stain. Low power.

A = bundle tissue; B = fat cells separating bundle from right side of septum fibrosum; C = left ventricular endocardium covering septum fibrosum.



Gross and Fried

Lesions in Conduction System in Rheumatic Fever

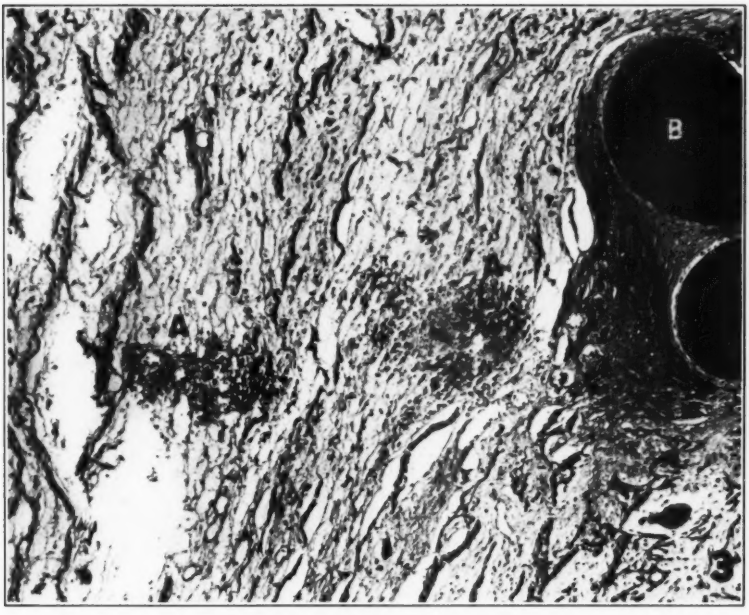
PLATE 5

FIG. 3. Cross-section of horizontal conduction system from case of active rheumatic fever (first attack). Age 10 years. Hematoxylin and eosin stain. Low power.

A = Aschoff bodies in edematous and inflamed bundle tissue; B = large blood vessels (injected) of bundle showing inflammatory cells in adventitia.

FIG. 4. Cross-section of horizontal conduction system from case of active rheumatic fever (first attack). Age 17 months. Hematoxylin and eosin stain. Low power.

A = inflamed and edematous bundle tissue. Note compression of cells; B = markedly inflamed septum fibrosum. Note marked increase in capillaries and inflammatory cells; C = zone of marked inflammation with arterioles; D = concentration of inflammatory cells between bundle and septum fibrosum.


A
B

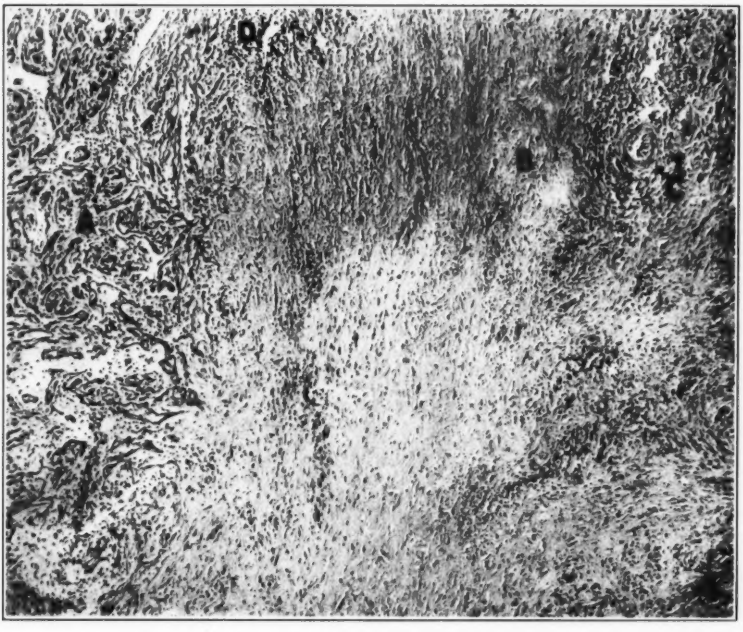


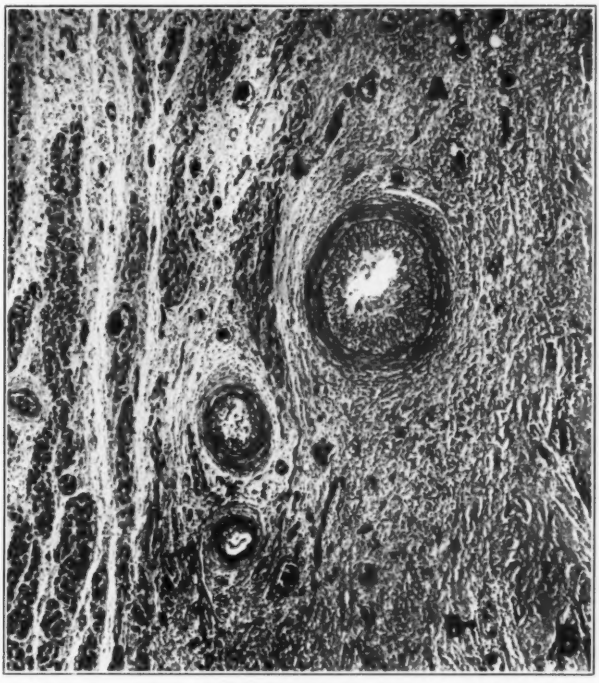
PLATE 6

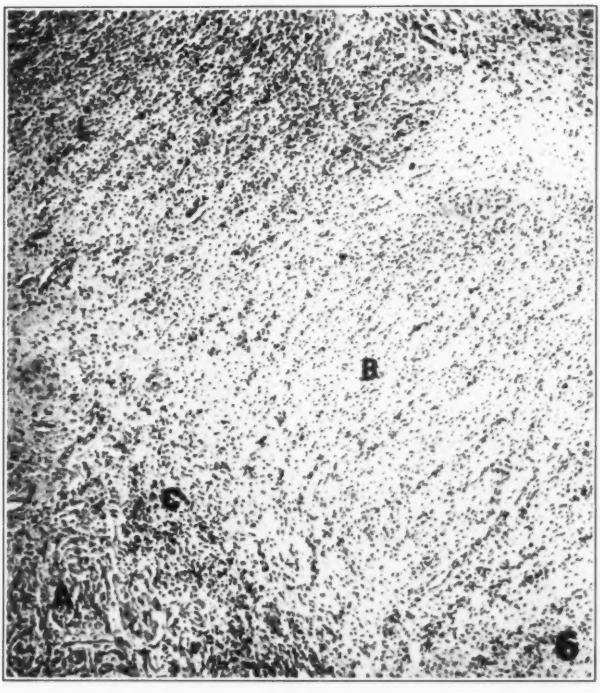
FIG. 5. Cross-section of horizontal conduction system from case of active rheumatic fever showing typical musculo-elastic hyperplastic vessels. Age 9 years. Weigert's elastic and Van Gieson's connective tissue stain. Low power.

A = marked inflammation with engorged capillaries; B = arteriole in inflamed septum fibrosum.

FIG. 6. Cross-section of horizontal conduction system from case of active rheumatic fever (first attack). Age 17 months. Hematoxylin and eosin stain. Low power.

A = inflamed bundle; B = septum fibrosum permeated with inflammatory cells and capillaries of the granulation tissue type; C = concentration of inflammatory cells between bundle and inflamed septum fibrosum.





Gross and Fried Lesions in Conduction System in Rheumatic Fever

BENIGN AND MALIGNANT HYPERTENSION AND NEPHROSCLEROSIS *

A CLINICAL AND PATHOLOGICAL STUDY

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INTRODUCTION

In the classification of Bright's disease there still exists much confusion. The number of historical reviews on the subject makes it unnecessary to discuss in detail this aspect of the question. Our objective has been a clinical and pathological study of the renal manifestations of arterial hypertension in an endeavor to reach a clearer understanding of the conditions commonly labeled "benign and malignant hypertension or nephrosclerosis."

Owing to the reciprocal relationship of hypertension and kidney disease the later stages of both conditions often present great difficulty in differentiation to both pathologist and clinician. It is, therefore, essential to describe in detail the histological criteria we employed in excluding those renal conditions which produce "secondary hypertension." Disregard of these considerations by many authors is to a great extent responsible for the difficulty in reconciling the various classifications of Bright's disease.

Criteria Used for Differential Diagnosis

The outstanding conditions to be discussed are diffuse glomerulonephritis and ascending processes leading to contraction of the kidney.

(*1*) *Diffuse Glomerulonephritis:* In making the diagnosis of diffuse glomerulonephritis the most important feature is the diffuseness of the glomerular lesion. In the acute and subacute stages the clinical and histological pictures are sufficiently characteristic to make the diagnosis free from doubt. It is in the chronic stage — the so-

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called secondary contracted kidney — that difficulties arise. The past history of the patient is in many instances incomplete or reveals no evidence of a preceding attack of acute nephritis. Hypertension with variable albuminuria over a period of years is often the only manifestation. Histologically the glomerular lesion may not be an entirely diffuse one — the percentage of diseased glomeruli frequently not exceeding 60 to 70 per cent. Furthermore, secondary arterial and arteriolar changes may in the later stages complicate the picture to such an extent that it is impossible to decide whether the glomerular or vascular lesion predominates. We have excluded cases of this type for reasons which will be discussed later. The gross appearance of the kidney in diffuse glomerulonephritis, though in many cases characteristic, is subject to such wide variations that it is an unreliable criterion in differential diagnosis. In short, we based the differential diagnosis of glomerulonephritis on a predominantly diffuse glomerular lesion, especially in cases where the clinical data were inconclusive.

(2) *Ascending Contraction of the Kidney:* This often presents still greater difficulty in recognition. Here again the clinical history may afford little or no assistance. Histologically the main diagnostic feature is the interstitial infiltration which is usually, but not invariably, most marked in the medulla. The macroscopic appearance of the kidney may be more characteristic. Widening of the calyces, thickening, hyperemia and dullness of the mucosa may suggest the ascending nature of the process, while the breadth and flatness of the cortical scars indicate that the contraction originated in a large group of collecting tubules, thereby involving a wide zone of renal substance. It has to be emphasized that widening of the renal pelvis is not invariably present and in long-standing cases the gross appearance may be so indefinite that the diagnosis has to be made on histological grounds. Unfortunately, it is in this type of case that secondary vascular changes tend to be most severe and the picture may be so complicated as to make a definite decision impossible. Certain histological features are, however, characteristic. The distribution of the cellular infiltration has been mentioned. Its character is of importance — the presence of plasma cells, monocytes and leukocytes enabling us to discriminate between the inflammatory and purely ischemic scarring processes. Leukocytic cylinders are frequently encountered, particularly in the collecting tubules. Most

remarkable is the relative infrequency of inflammatory changes in the glomeruli. There may be thickening of the glomerular capsule, usually associated with some degree of atrophy, such atrophied glomeruli being characteristically crowded together in scarred areas from which the tubules have disappeared.

A picture closely resembling the above may be encountered in the condition Fahr [1] has termed "incomplete infarction" in which circulatory insufficiency causes atrophy of the tubules while the glomeruli for the most part remain intact. The finding of an old arterial thrombosis or very severe arteriosclerosis in the artery of supply points to the diagnosis, but in the absence of such indication the character of the interstitial infiltration must be taken as a guide. In the resorptive scar tissue of incomplete infarction inflammatory infiltration of the type described above is absent. The whole available evidence — clinical, macroscopic, and microscopic — must therefore be considered in making the exclusion diagnosis of old ascending processes.

We have briefly referred above to examples of extreme contraction of the kidney in which vascular and glomerular lesions are inextricably mixed. To these may be added advanced cases of ascending contraction. All such instances represent the final stages of a disease whose early origin it is impossible to recognize with certainty. They constituted only a very small group in our series and we felt it justifiable to exclude them on the above grounds.

Benign Hypertension, Benign Nephrosclerosis and Malignant Nephrosclerosis (Fahr [1])

We have discussed above the method of exclusion of cases of glomerulonephritis and ascending contraction. The remaining cases appear under a somewhat confusing variety of terms. Histologically the kidneys show all degrees of arterial and arteriolar changes of different types overshadowing any glomerular and tubular lesions which may be present. Clinically renal involvement may or may not be evident. According to Fahr these cases would fall into three groups — essential hypertension, benign nephrosclerosis and malignant nephrosclerosis. Since a critical analysis of this classification has been our special objective, it is desirable to outline the general conceptions involved. As "essential hypertension" Fahr designates cases in which renal vascular changes are absent. When the kidneys show arterial and arteriolar sclerosis the term benign nephrosclerosis

is used and the hypertension is considered to be secondary to the renal vascular changes. The differentiation of essential and benign renal hypertension is therefore made on the basis of a quantitative estimation of the diffuseness of arterial involvement (Fahr [2]). Histologically there is no clear line of demarcation between benign nephrosclerosis and Ziegler's "Arteriosclerotische Schrumpfniere," that is, circulatory atrophy without hypertension, but a presumption in favor of the former may be made on the basis of a diffuse arteriolar sclerosis.

As a subgroup of benign nephrosclerosis Fahr has described a series of cases showing histologically focal glomerulitis. On the basis of these lesions and the presence of elevated non-protein nitrogen in the blood in such cases, Fahr regards this as a decompensated form of benign nephrosclerosis. Other observers (Volhard,[3] Lichtwitz [4]) consider that a true renal decompensation cannot be recognized clinically and maintain that cardiac failure is chiefly responsible for the nitrogen retention (see page 66).

The malignant nephrosclerosis of Fahr is characterized by specific arterial lesions in the kidneys, namely, productive endarteritis and necrotizing arteriolitis. The latter change is considered of greater diagnostic value since the former may occasionally be absent. Focal glomerular lesions are present similar to those found in decompensated benign nephrosclerosis but are usually more severe in character and extent. The tubules commonly show degenerative changes. The hypertension is regarded as secondary to the arterial and arteriolar lesions which in turn are believed to result from the action of an exogenous toxin. Characteristically the lesions are fairly diffuse in the kidney and may be present in other organs, especially those of the splanchnic area. The vascular necrosis with reactive, exudative and proliferative changes resembles in its most marked form the condition known as periarteritis nodosa, though the latter usually affects larger vessels and has a wider organ distribution.

As Volhard points out, it has become an urgent clinical necessity to establish criteria for the differential diagnosis of benign and malignant hypertension. Fahr has given us certain criteria for making the differentiation histologically. The reasons why Volhard and Fahr's conception of malignant nephrosclerosis as a definite disease entity has not received universal acceptance are twofold. First, there exist many borderline or transitional cases which give rise to great diffi-

culties in classification; second, the "specific" arterial changes are frequently encountered in other conditions, such as diffuse glomerulonephritis, where their rôle as primary lesions can obviously not be maintained. To these criticisms we have directed our attention — in particular examining the controversial "borderline" cases from a clinical and histological standpoint. We have come to the conclusion that these cases are of vital importance, not only as a criticism of the classifications presented by Volhard and by Fahr but more especially in giving a clearer conception of the nature and course of the malignant type of hypertension.

General Histological Changes

A. Arteries

For rough comparative purposes we made a distinction between large, medium-sized and small arteries and studied the lesions in each. In conformity with the majority of observers we considered as small vessels (arterioles) all sizes up to the interlobular arteries. We differ in this respect from Bell and Clawson,[5] who would confine the term arteriole to the vasa afferentia. Arterial changes were studied not only in the kidney but in other organs, especially the pancreas and adrenals.

(*1*) *Large and Medium Sized Arteries:* Passing over the common form of arteriosclerosis we would call attention to one special form of it, the so-called productive endarteritis, especially to emphasize the difficulties that may arise in differentiating it from pure arteriosclerosis or "elastosis" of Volhard. Between the two, all transitional stages are encountered. In clear-cut cases of productive endarteritis degenerative changes are absent from the media, which tends rather to undergo muscular hypertrophy. The subintima is nucleated and has the appearance of "onion layers." In this pure form there is no difficulty in distinguishing the lesion from fully developed degenerative arteriosclerosis. Secondary degenerative changes may, however, occur in endarteritis, for example, mucoid and hyaline material may appear in the intima, in which case the latter has a less nucleated appearance. There is no clear line of demarcation between this picture and that of a purely degenerative arteriosclerotic change. Since both types of lesion may occur in different stages in the same kidney it may be almost impossible to make a definite decision.

In taking productive endarteritis as a criterion for the provisional diagnosis of malignant nephrosclerosis we accept, therefore, only those cases in which it occurs diffusely or at least in which it is the predominating arterial lesion. Focal endarteritis, especially of the transitional type described above, is a fairly common finding in scarred kidneys and is of no diagnostic value.

(2) *Small Arteries:* It is unnecessary to comment on the common arteriosclerotic changes — hyalinization and fatty degeneration. One point should be emphasized — the frequency of severe arteriosclerosis in the suprarenal glands is given surprisingly little notice in the literature, yet we found that this organ was affected with about the same degree of severity as the pancreas. In the case of the suprarenal the arteriosclerosis is predominant in the arteries of the periadrenal fatty tissue from which the gland receives the blood supply.

We have, furthermore, occasionally encountered a peculiar type of staining reaction with eosin-methylene blue which appears to be of some significance. The arteriolar wall takes on a fairly diffuse bluish appearance contrasting strongly with the bright red hyalinized arterioles. In a severer form the vessel wall may give a homogeneous dark blue stain (Fig. 1), or the dark staining material may appear as cloudy bluish masses, or again as sharply defined flakes (Fig. 2). The latter appearance is particularly common in the periadrenal fatty tissue. The exact significance of this change is uncertain. It is most frequently seen where arteriosclerosis is severe and is often associated with true arteriolonecrosis. We did not feel that it could be regarded as an acute necrosis in Fahr's sense since disintegration of the vessel wall, invasion by red blood cells and exudative and proliferative changes were absent. We termed it "fibrinoid degeneration," concluding that in rate of development and severity it probably occupies an intermediate place between arteriolar necrosis and hyalinization.

The term "necrotizing arteriolitis" (Fahr) is used to bring this type of lesion into contrast with hyalinization of the arterioles, and is employed on account of the frequent association of the arteriolar necrosis with an inflammatory reaction, which is never found in hyalinization.

In small, medium and large arteries respectively, we attempted to make a rough quantitative estimate of the severity of the arteriosclerotic process for comparative purposes. Taking into account the

differences of distribution, as well as the degree of change in the individual vessels, four degrees of severity were arbitrarily recognized for each vessel group and expressed numerically (1–4). The sum of these figures for all three vessel groups is taken as representative of the degree of arteriosclerosis in the kidney under examination.

B. Glomeruli

(*1*) *Purely Degenerative Changes:* The processes of atrophy and hyalinization of the glomeruli in kidneys showing arterial or arteriolar sclerosis do not require detailed consideration. Their modes of development have recently been reported in a paper by one of us.[6] An occasional finding of some interest is a diffuse and very striking thickening of the intercapillary connective tissue. A detailed study of this condition will be presented in a separate communication.

(*2*) *Inflammatory Changes:* (Figs. 3–6) Inflammatory lesions of the glomeruli in cases of hypertension associated with renal vascular disease are subject to wide differences in interpretation. In Fahr's malignant nephrosclerosis, glomerulitis, though essentially focal in distribution, may be so severe and extensive that some observers regard it as a primary glomerulonephritis.[21] Cases of benign hypertension showing glomerular changes of this type are much less frequent and the lesions relatively scanty, so much so in fact that occasionally a careful search reveals only two or three affected glomeruli in each section. Their existence, however, is of sufficient importance to merit a detailed description, especially since Fahr emphasizes the proliferative character of the lesion which in our cases has been minimal. The earliest change to be observed is a swelling of the epithelial cells of the glomerular loops. The cells may present a honeycomb appearance which is occasionally attributable to the deposition of fat droplets. The capillary basement membrane may undergo slight degenerative changes, giving it an irregular appearance, and collapse of the capillary makes the affected loop stand out from the remaining patent capillaries of the glomerulus. In other glomeruli the cytoplasm of the enlarged epithelial cells takes on a bluish stain and the nuclei undergo fragmentation. Early fibrinous adhesions to the parietal layer of Bowman's capsule may be present. In later stages there is definite broadening of the capillary wall, which takes on a turbid, finely granular, purplish red appearance with blurring of its

outline. The nuclei become irregular and pyknotic and at the center of the glomerular loop disappear entirely. At the periphery, especially at the site of adhesions, nuclear proliferation may be seen, but increase in polymorphonuclear leukocytes is very rare. Occasionally areas of capillary collapse give the impression of an increase in nuclei, but the phenomenon is more probably a crowding effect. In still later stages the affected loops take on a homogeneous appearance resembling hyalinization which may be focal or may involve the whole glomerulus. This picture is very characteristic and the eosin-methylene blue stain reveals a pinkish, homogeneous hyaline area containing sharply demarcated, flake-like masses which have a vivid red or bluish hue. The fat stain reveals much neutral fat and lipoids in these areas of "hyalin necrosis," and doubly refractile substances are frequently present.

These glomerular lesions are principally of an "alterative" nature but according to the severity of the process may be followed by exudative or proliferative changes in varying degree. Even in the presence of the latter, however, the primary necrotizing process is always evident. On this account we applied the term "alterative glomerulitis" and contrast the lesion with that of acute diffuse glomerulonephritis in which the primary alterative changes can be demonstrated only with difficulty.

Other forms of glomerulitis, such as are produced by embolic processes, acute suppurative changes, periglomerular infiltrations and agonal fibrin thrombi, are occasionally encountered but their presence is so obviously incidental that no further mention is considered necessary.

C. Tubules

The common regressive changes, albuminuric degeneration, hyaline droplet degeneration, necrosis and fat deposition do not demand detailed consideration. Their occurrence is common to all types of arteriosclerotic kidneys, although hyaline droplet degeneration is more frequently seen in the malignant type than in the benign type of nephrosclerosis. The features to which we pay special attention are tubular dilatation and hyperplasia. It is a rather obvious assumption that tubular dilatation is associated with decrease of kidney function, but we have been unable to find any comparative study of the relationship from the clinical and histological aspects.

Jores [7] distinguishes different forms of tubular hyperplasia. Among these are (1) dilatation of the lumen with enlargement of epithelial cells, (2) lateral sprouting and (3) prolongation of tubules — recognizable by the increase in number of tubular cross-sections relative to the number of glomeruli. We confined our observations to types (1) and (2) and recognized four degrees of dilatation—slight, moderate, considerable and severe. The first degree may perhaps be described as questionable; the last, so commonly found in chronic glomerulonephritis, is rarely to be seen in arteriosclerotic contracted kidneys. We realize that this method is only roughly quantitative and open to subjective errors. Nevertheless, it is in our opinion the only reliable morphological guide to functional impairment. The degree of kidney shrinkage in the individual case may be misleading, although the average kidney weight in severe arteriosclerosis is subnormal. Histologically resorptive scar tissue and hyalinized glomeruli may give a false impression of the extent of reduction of kidney parenchyma. The severity of the arteriosclerosis may be an equally unreliable guide to impairment of function, since it has been shown by perfusion experiments (Kimmelstiel [8]) that no exact parallelism exists between the degree of arteriosclerosis and diminution of blood flow through the kidney. Tubular dilatation with hyperplasia, on the other hand, is a direct response to reduction of kidney parenchyma below the functional reserve, and for this reason has received our special attention. According to the rate and distribution of the scarring process tubular dilatation may be focal or more or less diffuse, and this variation has to be considered in estimating its degree of development. It is furthermore a common observation, and one for which we have no adequate explanation, that in early cases tubular dilatation is confined to the periphery of the cortex immediately below the kidney capsule.

Clinical Investigations

(*a*) *Hypertension:* Elevation of blood pressure was the basis of selection of our cases. As to the duration of the hypertension our information is naturally incomplete in many instances. Moreover, on the final admission to the hospital a number of the cases showed slight or absent elevation on account of heart failure or coronary thrombosis. There was, however, in such cases a well authenticated

previous history of hypertension, or an increased heart weight with left ventricular hypertrophy but without valvular lesion was found at autopsy.

(*b*) *Renal Function Tests:* Chief emphasis has been placed on urine concentration tests and the level of the non-protein nitrogen of the blood. Volhard's dilution-concentration tests are available in many instances but in their absence repeated routine specific gravity tests have been accepted. Any inaccuracy arising from this source is in a positive direction, that is, values for concentrating power, obtained from repeated specific gravity tests on routine specimens, tend to be higher than those given by the concentration dilution test. Abnormal urinary constituents — albumin, erythrocytes, leukocytes and casts — have been noted. Other tests of renal function, creatinine clearance, urea clearance and the phenolsulphonephthalein tests have been studied, but as these are not available in the majority of cases, they are used only as supporting evidence and have not been employed for comparative purposes.

(*c*) *General Features:* Particular attention was paid to the age of the patient, past history of renal disease, scarlet fever or toxemia of pregnancy. Details of ophthalmoscopic examination of the retina were obtained whenever possible. We studied with special care the terminal manifestations of the disease. In cases of hypertension with renal involvement "uremia" is often reported as a cause of death. Regarding uremia as a complex syndrome with renal, cerebral and cardiac elements we feel that the term as such is misleading and, if used, requires strict definition. This is especially important in "malignant" hypertension, and we shall elaborate the point when discussing these cases.

Analysis of Clinical and Histological Data

Our material was drawn from consecutive clinical and autopsy records for 1932, 1933 and 1934.* All cases showing evidence of hypertension were investigated. From these, diffuse glomerulonephritis, ascending contraction and other obviously renal conditions were eliminated by the methods discussed above. The remaining series, in all 250 cases, were provisionally regarded as vascular or

* From the Mallory Institute and Second and Fourth Medical Services of the Boston City Hospital.

"essential" hypertension. There was in these cases no evidence of antecedent renal disease. A provisional separation of this series into benign and malignant groups was made on the basis of Fahr's criteria, *i.e.* productive endarteritis and necrotizing arteriolitis in the kidney. Borderline cases showing these arterial changes in atypical form or distribution were placed in a separate class, the significance of which is discussed under the malignant group. For reasons which will become obvious later, we have used the clinical expressions benign and malignant hypertension, rather than the morphological term "nephrosclerosis."

A. Benign Hypertension

A careful analysis of the degree of renal involvement in these cases was first made from the clinical and histological aspects in an attempt to substantiate or disprove Fahr's conception of a true renal decompensation in benign hypertension. On the basis of this analysis the cases fall into four groups. The findings are summarized in Table I.

(*1*) *Benign Hypertension with No Renal Involvement:* This is the largest group, constituting some 60 per cent of the whole. Clinically, the concentrating power of the kidneys is within normal limits, and there is no elevation of non-protein nitrogen of the blood. Histologically the kidneys of this group show no tubular dilatation and no glomerulitis. Arteriosclerosis is present in all degrees with an average comparative figure of $4\frac{1}{2}$.

(*2*) *Benign Hypertension with "Extrarenal" Nitrogen Retention:* (15 per cent of cases.) Elevation of non-protein nitrogen is in these cases attributable to diminished blood flow through the kidney arising from cardiac failure or some other extrarenal cause. Concentrating power is unimpaired, however, and histologically there is no tubular dilatation and no glomerulitis. The degree of arteriosclerosis is essentially the same as in Group 1.

(*3*) *Benign Hypertension with Renal Impairment:* (10 per cent of cases.) These cases show the early stages of renal involvement. The non-protein nitrogen of the blood is within normal limits but concentration-dilution tests show slight to moderate impairment of function, the maximum specific gravity being 1021. Histologically we find slight to moderate tubular dilatation but no glomerulitis. Ar-

teriosclerosis is more severe than in Groups 1 and 2 with a comparative figure of 6½.

(*4*) *Benign Hypertension with Renal Decompensation:* This group constitutes 11 per cent of the cases. The main histological features are focal alterative glomerulitis of the types already described, considerable tubular dilatation and severe arteriosclerosis. Clinically the concentrating power of the kidneys is impaired, specific gravities of urine in the Volhard test falling between 1012 and 1017. The

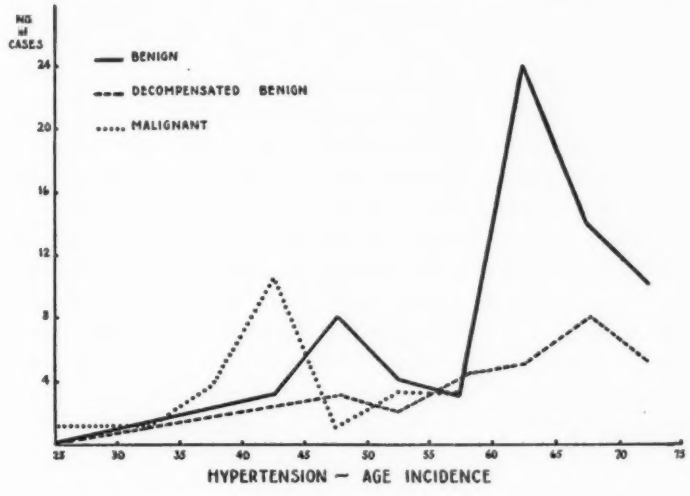
TABLE I

Renal Involvement in Benign Hypertension and Kidney Changes

Groups	Percentage	Average specific gravity of urine	Limits of N. P. N.	Average arteriosclerosis	Tubular dilatation	Glomerulitis
1. No renal involvement	60	Within normal limits	Within normal limits	4½	Negative	Negative
2. Extrarenal nitrogen retention	15	Within normal limits	50–135	4	Negative	Negative
3. Renal impairment	10	1012–1021	33–60	6½	Slight to moderate	Negative
4. Renal decompensation	11	1012–1017	57–200	8	Moderate to considerable	Present

non-protein nitrogen of the blood is elevated in all cases except two, the limits being 57–200 with an average value of 73 mg. per 100 cc. It is necessary to review the evidence which convinced us that this is a true renal decompensation occurring in the course of benign hypertension. In the first place the clinical picture up to the stage of renal failure is that of other cases of benign hypertension (Group 1). Uremia plays a subordinate part in the terminal picture, the common causes of death being cardiac failure, coronary thrombosis, cerebral accidents or bronchopneumonia. In a few cases, however, true uremic coma was undoubtedly present. The most characteristic clinical feature is the age group into which these cases fall. When the age incidence is compared with that of Group 1 it is seen that the peak falls several years later (Text-Fig. 1). Histologically the relation of these cases to the previous groups of benign hypertension is even

more clearly brought out. As we pass from Group 1 through the stage of renal impairment to that of insufficiency there is a striking increase in the severity of arteriosclerosis. As seen in the summarizing table (Table I), Groups 1 and 2 show the same average degree of arteriosclerosis (4–4½), Group 3 an intermediate figure (6½) and Group 4 a still severer degree (8). This gradation in kidney involvement receives strong support from the existence of Group 3, in which tubular dilatation and impaired concentrating power are alone pres-



TEXT-FIG. 1

ent. When the average kidney weights are similarly compared, the decompensated group shows a greater shrinkage than the cases with no clinical or histological evidence of renal involvement (256 and 326 gm. respectively). We are led to the conclusion, therefore, that if the patient lives long enough the arteriosclerotic process leads eventually to diminution in kidney parenchyma below the limits of its functional reserve. The high percentage of cases with renal decompensation in our series is probably attributable to the relatively late age incidence of our benign hypertensive groups as a whole as is seen from Text-Figure 1. The evidence presented above shows clearly that we are dealing with true renal decompensation as Fahr maintains, and not merely with a nitrogen retention due to terminal

heart failure. The existence of Group 2 with no histological evidence of renal impairment but with extrarenal nitrogen retention emphasizes this point. Lastly, these cases cannot be regarded as malignant hypertension. They fall into a much later age group, they lack the fulminating element of malignant hypertension, hypertensive retinopathy is never observed, and histologically the "specific" arterial lesions of the latter are absent. To Fahr's distinction between essential hypertension and benign nephrosclerosis the above analysis lends no support but rather emphasizes the unity of the whole group of benign hypertension as a primary generalized vascular disease.

B. Malignant Hypertension

We have now to consider those cases which were separated from the main group on account of "specific" arterial lesions — necrotizing arteriolitis or endarteritis. The classical picture of malignant hypertension presented by Volhard and Fahr is that of unusually high blood pressure occurring in relatively young subjects and having a rapid termination in uremia. Hypertensive retinopathy (edema of the disc and retina, with retinal exudates)* is a constant feature. Histologically necrotizing arteriolitis is found in the kidney and in other organs, usually associated with productive endarteritis. Focal glomerulitis is invariably present. Twelve of our cases fall into the above group. We have, however, made a careful study of the borderline cases previously referred to in which there are discrepancies from the classical picture described above. These cases have led us to the conclusion that the malignant nephrosclerosis of Volhard and Fahr does not give a true picture of the disease "malignant hypertension" but represents only the renal end-stage. Cases exist in which malignant hypertension is present but death occurs before this renal end-stage is reached.

As in benign hypertension, the malignant group can be arranged on clinical and histological grounds in increasing order of renal involvement. Such an arrangement has been made in Tables II and III. Owing to the fulminating nature of the disease, cases with *no renal involvement* are very rare. In the first group of cases, however, (Table II) renal failure plays little or no part in the final picture. Clinically the diagnosis of malignant hypertension is supported by the relatively young age incidence, the unusually high blood pres-

* The term hypertensive retinopathy is used in this sense throughout the paper.

sure, the presence of hypertensive retinopathy, and the fulminating termination. In certain cases the actual cause of death was obscure, being termed clinically "hypertensive encephalopathy" (*vide infra*) and at autopsy no organic lesion could be demonstrated except in one case which showed multiple small hemorrhages in the cerebral cortex.

Referring again to Table II, there was little or no elevation of non-protein nitrogen of the blood in these cases. Renal impairment, as indicated by diminished concentrating power of the urine, was slight or absent.

The small extent of clinical involvement of the kidney is paralleled in the histological picture. Tubular dilatation is either absent or slight compared with Group II. Glomerulitis is absent in Case 1, and only one instance was found in several sections in Case 4. The remaining members of the group show scanty focal glomerulitis compared with the more extensive changes in Group II. The same holds true for necrotizing arteriolitis. This lesion is absent in Cases 4 and 8. In Case 2 it is confined to the pancreas and adrenal and in the remainder its distribution is so scanty that only occasional examples are seen in going through several sections. Productive endarteritis is found as a diffuse lesion in only two cases and is absent or focally distributed in the remainder.

Finally, Cases 9 to 19 (Table III) show the *fully developed picture with uremia*. Histologically the findings are characteristic and it is to be noted that in this group necrotizing arteriolitis, endarteritis and focal glomerulitis are as a rule far more severe in extent than in the previous cases.

The separation of the above cases into groups is somewhat artificial. They show a gradual increase in kidney involvement. The arrangement is made solely to bring out the relation of malignant hypertension as such to the end-stage which has been termed malignant nephrosclerosis. According to Fahr's criteria cases of the first group showing no necrotizing arteriolitis cannot be regarded as malignant nephrosclerosis, but we feel that their clinical course warrants the diagnosis of malignant hypertension — a malignant hypertension which proves fatal from extrarenal causes before the kidneys are seriously involved.

As we pass from Group I to Group II, the extensiveness of the lesions in the kidney increases and the individual elements, which at

TABLE II

Malignant Hypertension. Group I

Case No.	Autopsy No.	Age	Blood pressure	N. P. N.	Specific gravity	Retinopathy	Glomerulitis	Productive Endarteritis		Necrotizing Arteriolitis		Tubular dilatation	Cause of death
								Kidney	Other organs	Kidney	Other organs		
		yrs.	*mm. Hg.*										
1	31–68 Ch.-H	12	260/160 200/150	Normal	1005–1020	+	Negative	Diffuse	Present	Very sparsely	Negative	Negative	Septicemia
2	32–231	45	220/130	24	1004	+	Occasional	Negative	Negative	Negative	Present in pancreas and adrenal	+	"Encephalopathy"
3	32–341	52	245/120 270/130	45–29 42–43	1010–1022	+	Occasional	Focal slight	Present in pancreas and adrenal	Negative	Negative	++	Bronchopneumonia, cerebral softening
4	33–10	24	220/140 245/140	35–56 41	1006–1022	+	Only 1 found	Moderate	Negative	Negative	Negative	++	"Encephalopathy"
5	33–264	45	260/150 240/150	30–34	1008–1022	+	Very few	Slight in some small arteries	Negative	Negative (occasional fibrinoid degeneration)	Adrenal (fibrinoid degeneration)	+	"Encephalopathy"
6	33–714	40	230/150 180/110	33–66	1010–1014	+	Occasional	Some, focal slow in type	Negative	Occasional	Occasional (fibrinoid degeneration)	++	Multiple punctate hemorrhages in brain
7	34–U20	36	260/140	23–50	1003–1009	+	Occasional	Diffuse	Adrenal severe	Very sparsely	Negative	+++	Adrenalectomy 3 weeks ago. Pulmonary embolism
8	34–84	41	250/150	33	1008–1012	+	Occasional	Some, focal slow in type	Pancreas adrenal fairly diffuse	Negative	Negative	+++	Bronchopneumonia

TABLE III

Malignant Hypertension. Group II

Case No.	Autopsy No.	Age	Blood pressure	N. P. N.	Specific gravity	Retinopathy	Glomerulitis	Productive Endarteritis		Necrotizing Arteriolitis		Tubular dilatation	Cause of death
								Kidney	Other organs	Kidney	Other organs		
		yrs.	*mm. Hg.*										
9	32–88	42	220/110	80 248	1007–1010	+	Occasional	Diffuse	Present in pancreas	Occasional	Present in pancreas	++$^{+}$	Uremia
10	32–271	29	260/150	33–35 21–120	1005–1018	+	Frequent	Occasional		Extensive		++$^{+}$	Uremia
11	32–533	45	235/120	82–190 265	1010–1016	+	Occasional	Diffuse	Present in pancreas	Negative	Negative	+++	Uremia
12	33–64	41	260/150	205 190	1010–1012	+	Frequent	Considerable	Negative	Fairly diffuse	Negative	+++	Uremia
13	33–327	45	200/120 190/120	175 165		+	Frequent	Diffuse	Present	Frequent	Present in pancreas	++	Uremia
14	33–440	43	215/155	45–67 53	1004–1009	+	Occasional	Occasional	Present in pancreas	Extensive	Negative	+++$^{+}$	Bronchopneumonia
15	33–514	46	250/140	?	1005–1016	+	Frequent	Diffuse	Present in pancreas	Extensive	Negative	+++	Encephalopathy ? Uremia ?
16	33–722	52	230/130	175	1015–1018	+	Occasional	Diffuse	Negative	Occasional	Negative	++++	Uremia
17	33–727	40	240/170 260/170	35–41 86–207	1006–1015	+	Occasional	Diffuse	Present	Extensive	Present only in cecum	+++	Uremia
18	34–388	41	200/120	150	1012	+	Frequent	Not diffuse	Negative	Extensive	Negative	+++$^{+}$	Uremia
19	S–34–3225	46	220/130	133	?	+	Occasional	Fairly extensive	?	Fairly extensive		+++$^{+}$	Uremia

first are scanty and irregular in distribution, combine more and more to form the histological entity or "full blown picture" of malignant nephrosclerosis. Thus, the histological diagnosis of this condition does not depend on any single specific lesion but can be made only from a consideration of the whole histological picture.

Concerning the nature of the nitrogen retention in malignant nephrosclerosis, the same arguments which were employed in benign hypertension with renal decompensation apply to the malignant type. Impaired concentrating power and tubular dilatation point to a true renal origin so that cardiac failure is only secondarily responsible, if at all, for elevation of the non-protein nitrogen of the blood.

So far we have regarded benign and malignant hypertension as two different processes, the former incident in relatively old individuals associated with varying degrees of arteriosclerosis and in some cases gradually leading to moderate renal insufficiency, the latter occurring in younger subjects and having a fulminating termination usually involving the kidney, which shows characteristic arterial lesions. The difference may be considered as one in the reaction of the arteries of the individual to the vasospastic process, young subjects being more "reactive" than older individuals.

There is a second group of *borderline or non-classical cases* which supports this idea and also might answer the question whether "benign hypertension ever becomes malignant." These cases (Table IV) represent a transition between the decompensated benign group and the malignant nephrosclerosis. Compared with the latter, they belong to a relatively later age period and have a less fulminant progress. One case showed hypertensive retinopathy characteristic of the malignant hypertension. Renal impairment is present with moderate nitrogen retention, but the "uremic process" as such plays a less conspicuous part in the terminal picture — recalling in this respect the cases in Group I. Endarteritis is found associated with arteriosclerosis and is often of the transitional type which has been described. Necrotizing arteriolitis, however, is only slight in extent being frequently associated with the change we have termed "fibrinoid degeneration." We feel that these cases represent a malignant type of hypertension incident in relatively old individuals, often superimposed on long-standing benign hypertension, and in this sense corresponding to the "Umschlag" of Volhard.

TABLE IV

Malignant Hypertension. Group III. (Transitional Group)

Autopsy No.	Age	Blood pressure	N. P. N.	Specific gravity	Retinopathy	Glomerulitis	Productive endarteritis		Necrotizing arteriolitis		Tubular dilatation	Cause of death
							Kidney	Other organs	Kidney	Other organs		
	yrs.	*mm. Hg.*										
32–553	62	220/140	95	1008	Not examined	Occasional	Some focal slow type		Very sparsely		+++$^{+}$	Uremia
34–406	55	180/100	67	1014–1020	Not examined	Occasional	Negative	Negative	Occasional	Present in pancreas	+++	Cardiac failure
34–612	62	180/100	58	1004–1010	Not examined	Occasional	Negative	Negative	Occasional	Negative	++	Cardiac failure
34–707	60	260/135	52–97	1012–1018	+	Occasional	Diffuse	Present in pancreas	Negative	Negative	++$^{+}$	Uremia

The summarizing classification of benign and malignant hypertension may therefore be outlined as follows:

A. Benign Hypertension	
(1) No renal involvement (2) Extrarenal nitrogen retention	75%
(3) Renal impairment	10%
(4) Renal decompensation (decompensated benign nephrosclerosis)	11%
Intermediate or Transitional cases ("Umschlag" of Volhard) less than 1%	
B. Malignant Hypertension	
(1) No renal involvement (2) Renal impairment (3) Renal decompensation — the "malignant nephrosclerosis" of Volhard and Fahr	4%*

Discussion

I. Benign Hypertension

A. Hypertension and Renal Arteriosclerosis

(1) *Special Significance of Arteriolar Changes:* Our observations confirm the work of other observers on the relation of arteriosclerosis to hypertension. Although a definite parallelism exists between the two, it is in many instances imperfect. Certain authors regard arteriolar sclerosis as "morphologically characteristic" of hypertension (Herxheimer and Schulz,[9] Fahr,[1] Bell and Clawson [5]), but it appears from our data that the relationship is equally true for the medium sized vessels. Arteriosclerotic changes in some cases predominate in the arterioles, in others in the interlobular vessels. Involvement of the arterioles alone is decidedly rare. In view of the variations in statistics one should regard with caution any attempt to attribute a diagnostic significance to arteriolar changes.

(2) *Hypertension as the Cause of Renal Arteriosclerosis:* Several factors prevent us from accepting such a statement unreservedly.

* In collecting cases of malignant hypertension, the records of all services of the Boston City Hospital were drawn upon. The percentage incidence is, however, based only on those cases from the 2nd and 4th Medical Services.

Cases of renal arteriolosclerosis without hypertension, although rare, have been described (Löhlein,[10] von Monakoff,[11] Fahr,[1] Kimmelstiel [8]). We also encountered a few examples of this type. Longstanding experimental hypertension may be produced in animals by section of the aortic nerves or by kaolin injections into the cisterna magna, but apparently arteriosclerosis does not follow such hypertension (Hamperl and Heller,[12] Nordmann [13]). Graybiel, Allen and White [14] have performed muscle biopsies on the upper and lower extremities of patients with coarctation of the aorta. They failed to find any significant difference in the arterioles although there is in such cases a much higher blood pressure in the arm than in the leg. Fahr has recently pointed out that in cases of severe renal arteriolosclerosis, these changes are absent from the vessels of the kidney capsule. Since both groups of vessels originate from the same artery and are subject to the same blood pressure, it appears that hypertension *per se* cannot be regarded as the cause of the arteriosclerosis. Fahr interprets this observation as supporting his theory of the renal origin of the hypertension. Such a distribution of the arterial lesions can, however, be equally well explained on the assumption that arterial strain is proportional to functional activity in the area of supply.

The conclusion is that hypertension may be considered as an accelerating factor in the development of arteriosclerosis as Aschoff maintains and Fahr to some extent admits, but the reaction of different vascular regions to the hypertension is not uniform and is determined in some way by the functional activity of the area supplied.

(3) *Renal Arteriosclerosis as a Cause of Hypertension:* We have already referred to cases of hypertension in which renal arteriolosclerosis is absent. There is considerable difference of opinion as to the frequency of these cases. According to Herxheimer and Schulz,[9] renal arteriolosclerosis is found in 97 per cent of cases of cardiac hypertrophy with hypertension. Bell and Clawson [5] place the figure at 90 per cent. Fishberg [15] states that intact kidneys are decidedly uncommon. Such numerical values are based on a purely quantitative estimation which is open to subjective errors. If mechanical obstruction to the circulation through the kidney is to be considered as a contributory factor to the hypertension, it has to be borne in mind that less than 50 per cent of cases show a completely diffuse arteriolosclerosis. Jaffé [16] believed that dilatation of the vas afferens,

which he frequently observed in association with very early degenerative changes in the glomerulus, pointed to a primary circulatory disturbance which reflexly produced the hypertension. This finding is, however, inconstant; hence the evidence cannot be regarded as conclusive. On the positive side animal experiments undoubtedly indicate that obstruction to the kidney circulation produces hypertension (Goldblatt *et al.*[17]). In human kidneys Kimmelstiel has shown by postmortem perfusion that in certain cases of benign hypertension an actual obstruction to the circulation exists. These cases are of the type we have described above as decompensated benign nephrosclerosis. In this group, therefore, where impairment of renal function is present, one may be justified in regarding the arteriosclerotic process in the kidneys as a factor in augmenting or maintaining the hypertension. In all other cases we must regard hypertension and arteriosclerosis as undergoing a parallel development as age advances.

(*4*) *Functional Disturbances in Arteriosclerotic Kidneys* (*Renal Decompensation*): This group has been recognized by various observers but different interpretations have been placed upon it. Schürmann and MacMahon [18] consider the cases as transitional to malignant nephrosclerosis. Murphy and Grill [19] similarly maintain that no clear distinction exists between the two groups. Under Volhard's influence most authors incline to the opinion that when renal failure occurs in benign hypertension we should consider the disease to have entered on the malignant phase or "Umschlag" (Volhard,[3] Lange,[20] Fishberg [15]). Exception is made to this interpretation in cases where nitrogen retention is attributable to cardiac failure and disappears as the cardiac condition improves. We hope the analysis of our data clarifies the situation by establishing the decompensated benign group as a distinct entity, and by bringing out its true relation to malignant hypertension. We have pointed out the clinical and histological features which differentiate the two conditions and have contrasted these cases with those of extrarenal nitrogen retention. There is no doubt that cardiac failure may be present and may be the precipitating cause of renal failure, but the primary and most important element is the condition of the kidney itself. Although this is admitted by some writers as a possibility (Volhard,[3] Lichtwitz,[4] Fishberg,[15] Lange [20]), the frequency with which it occurs is not appreciated.

The significance and pathogenesis of the glomerular lesions in decompensated benign nephrosclerosis remain to be discussed. Are these to be regarded as the cause or the effect of renal insufficiency, or are the two manifestations attributable to a common cause? The first possibility appears extremely unlikely on account of the focal nature of the glomerulitis and the very scanty distribution of the lesion in many cases. Considering the second possibility it is obvious that nitrogen retention as such does not produce alterative glomerulitis, as the control Group 2 with extrarenal nitrogen retention clearly demonstrates. Moreover, there exist occasional cases of decompensated benign nephrosclerosis with no evidence of elevated non-protein nitrogen. Functional impairment (as indicated by diminished concentrating power and tubular dilatation) is, however, invariable and in view of the overwhelming frequency with which nitrogen retention is present in cases with glomerulitis we cannot exclude renal impairment as an etiological factor. A consideration of the third possibility — that renal insufficiency and alterative glomerulitis are attributable to the same cause — may throw further light on the matter. Can ischemia be regarded as the common cause? The common arteriosclerotic lesion of the glomeruli, produced by slow arterial occlusion, is an atrophic degenerative change. If we postulate a sudden ischemic process or spasm as the cause of the acute necrosis observed in alterative glomerulitis, a corresponding change should be discernible in the tubules. In fact, the tubular apparatus is more susceptible to vascular damage than the glomeruli. Such changes are not found. At most we encounter hyaline droplet degeneration in the tubules. Russell [21] pointed out that glomerulitis occurs in the vicinity of acute renal infarcts. Klemperer and Otani [22] also reported necrotizing arteriolitis in the same location, and regard the finding as suggestive evidence of the ischemic origin of these changes. We have examined a series of renal infarcts and have identified examples of the alterative glomerulitis already described. The corresponding tubules, however, show no acute necrosis but rather a necrobiotic process with regeneration, an observation which prevents us from ascribing the glomerular or arteriolar lesions to pure ischemia. The occurrence of glomerulitis and arteriolitis in the vicinity of acute renal infarcts suggests, indeed, a more probable explanation of their pathogenesis. We cannot escape the conclusion that in this very situation diffusible toxins are produced by the breakdown

of kidney tissue. Toxic and ischemic factors combined may produce alterative glomerulitis. The possibility, therefore, remains that in decompensated benign nephrosclerosis retained products may act as toxins on glomeruli which are already damaged by severe arteriosclerosis. Cases with no nitrogen retention are difficult to explain on this basis but we might suggest that toxic substances may be retained before the non-protein nitrogen rises appreciably, or that, owing to the fluctuant nature of the latter, it may be normal at the time of the determination. In view of the inconclusive character of the evidence, however, we still entertain the possibility of an extrarenal toxin which may produce vascular spasm with coincident renal failure and alterative glomerulitis.

II. Malignant Hypertension and Malignant Nephrosclerosis

In the presentation of cases of malignant hypertension we have briefly outlined our conception of the relation of malignant hypertension to malignant nephrosclerosis and have pointed out that borderline cases which are difficult to explain on the basis of Fahr's classification are in reality essential to an understanding of the true nature of the disease. We must now consider the new interpretation to be placed on the specific vascular lesions in relation to the clinical picture, both from the diagnostic point of view and also with reference to the pathogenesis of the condition.

In the first place we have attempted to readjust the emphasis which hitherto has been laid on specific arterial lesions in the kidney and transfer it to the clinical picture of malignant hypertension. The clinical features, which in our opinion characterize this type of hypertension, are the relatively young age incidence, the unusually high blood pressure, the occurrence of hypertensive retinopathy and the fulminating progress of the disease, with cerebral manifestations, designated, for want of greater knowledge, "hypertensive encephalopathy." Such manifestations include a variety of symptoms, among which may be mentioned severe headaches and dizziness, disorientation and loss of memory, transient paresis and paresthesias, occasionally convulsions, visual disturbances and finally coma, which may occur in the absence of gross cerebral lesions or nitrogen retention. The presence of such a clinical picture, which has been called "pseudo-uremia" by Volhard, distinguishes these cases from the benign type of hypertension. The most important inference result-

ing from our investigation is that malignant hypertension as such precedes the stage of renal involvement — the proof being based on histological as well as clinical evidence. It appears that too much emphasis has hitherto been placed on the renal end-stage — malignant nephrosclerosis. The rigid histological criteria introduced by Fahr must therefore be relaxed and extended to embrace cases which on clinical grounds should be included in the group of malignant hypertension.

The Specific Arterial Lesions

We have already stated that the cases described under malignant hypertension were primarily separated on the basis of "specific" arterial lesions — productive endarteritis or necrotizing arteriolitis. The distribution of these lesions throughout the group, however, is not consistent with the significance attributed to them by Volhard and Fahr. We shall now attempt to elaborate a fuller conception of the nature of these changes.

(*1*) *Productive Endarteritis:* Diffuse endarteritis in the small or medium sized arteries of the kidney is an unmistakable diagnostic criterion of malignant hypertension. In several of our cases, however, it is either entirely absent or inconspicuous, so that it cannot be regarded as an invariable feature of the disease. We agree with Klemperer and Otani [22] that productive endarteritis should be regarded as an accelerated form of arteriosclerosis. The transitional type of lesion we have already described supports this view and makes it difficult to regard the change as inflammatory (Evans [23] and Fahr [1]). Productive endarteritis differs from the purely degenerative arteriosclerosis in one important respect however, namely, that in the diffuse form it is invariably associated with high blood pressure. We may, therefore, justifiably regard it as a response to a particularly severe type of hypertension. The rate of development of the endarteritic process may be extremely rapid. Weiss, Parker and Robb [24] described a case of malignant nephrosclerosis in which one kidney was removed at operation 67 days before death. Through their courtesy we have been able to examine sections of both kidneys and observed a striking increase in the extent and severity of productive endarteritis during this interval. We may state, therefore, that productive endarteritis may be absent in the early stages of malignant hypertension, develops rapidly during the course of the

disease, is present as a diffuse lesion in the kidney and other organs in fully developed cases, and in the diffuse form is never seen in the absence of hypertension. We cannot, however, explain the lesion simply as a reaction to a severe type of vascular spasm. Prinzmetal and Wilson [25] have shown that the vasospastic process in malignant hypertension is universally distributed. The incidence of endarteritis is, however, by no means universal — usually being confined to the organs of the splanchnic area. We therefore make the assumption that the vessels in certain regions are more susceptible to the vasospastic process, a susceptibility which may be in some way related, as we have already suggested in discussing arteriosclerosis, to the functional activity of the area of supply. This view is consistent with the observation made by Fahr that endarteritis and arteriolitis are not found in the vessels in the kidney capsule. It is necessary, however, to mention the paper by Kernohan, Anderson and Keith [26] who describe thickening of the arterial wall in the voluntary muscles in hypertension. It is surprising that similar investigations (Graybiel *et al.*[14]) in cases of coarctation of the aorta did not reveal any difference in the arterioles in the arm and leg. In order to explain this discrepancy we may point to the recent experiments one of us [25] has made in which it was shown that the mechanism of arteriolar constriction in generalized hypertension is apparently different from that in coarctation of the aorta. The former has to be interpreted as intrinsic vascular spasm whereas the latter has been shown to be vasomotor in origin. Graybiel, Allen and White [14] suggest a similar explanation although experimental support was not available at that time.

(*2*) *Necrotizing Arteriolitis:* Since necrotizing arteriolitis in the kidney may be slight or even absent in cases with malignant hypertension we cannot justifiably regard it as causing the hypertension. Arteriolitis differs from productive endarteritis in its relation to renal failure. Cases which show a fulminating uremic termination with gross nitrogen retention (Group II), in general show severe necrotizing arteriolitis. In the pre-renal stage, however (Group I), this feature is slight or absent. The extent and severity of the focal glomerulitis also show a parallel relationship with the renal function. The pathogenesis of the arteriolar necrosis presents a similar problem to that of the origin of alterative glomerulitis in decompensated benign nephrosclerosis. The inconstancy of the lesion prevents us from re-

garding it as the cause of renal insufficiency. Its occurrence with alterative glomerulitis in the vicinity of acute infarcts suggests the association of toxic and acute ischemic factors in its production. We have referred above to evidence of vascular spasm in malignant hypertension. Such spasm has been observed in the retinal vessels and its existence is indicated on the pathological side by cases of cortical necrosis of the kidney and by the occurrence of "Fleckmilz" and "Fleckpancreas" in malignant nephrosclerosis.

That spasm alone does not cause arteriolitis is evident from the absence of this lesion in Raynaud's disease and acute eclampsia where vascular spasm is undoubtedly present. Klemperer and Otani [22] suggest that endarteritis in larger radicles may produce ischemic damage in the corresponding arterioles, thereby precipitating arteriolar necrosis. Since, however, necrotizing arteriolitis occurs in the absence of endarteritis (see also our Case 2), these authors were led to subdivide malignant nephrosclerosis into two types: one in which arteriolar necrosis is assumed to follow endarteritis ("accelerated arteriosclerosis"), the other in which true necrotizing arteriolitis is regarded as the primary lesion. Schürmann and MacMahon similarly attempted to distinguish two forms of malignant nephrosclerosis — an exogenous and an endogenous form — on the basis of a difference in distribution of the arteriolar lesion. We feel that the evidence is insufficient to justify such distinctions. In the first place the separation of arteriolar necrosis and necrotizing arteriolitis is unjustifiable since transitions frequently occur from one to the other. Secondly there is no constant relation between endarteritis and arteriolar necrosis or arteriolitis. The distribution of the lesions in the arterioles is indeed very irregular and we feel cannot be regarded as an adequate basis for subdivision of cases. Such variations as occur are attributable to the different stages of development of the disease rather than to differences in pathogenesis. It is impossible to escape the fact that in malignant hypertension the development of both arteriolitis and glomerulitis is closely related to renal insufficiency. By analogy with decompensated benign nephrosclerosis it seems reasonable to regard the renal failure as the direct result of acute functional ischemia. Whether some toxin, producing the vascular spasm, produces also the glomerulitis and arteriolitis, or whether, alternatively, the combination of acute ischemia and retained toxic substances be regarded as the cause remains an open

question. Whatever their pathogenesis, these manifestations must be considered as essentially characteristic of the acute renal end-stage of malignant hypertension, independent of the endarteritis and in certain cases appearing before the latter has developed. When death occurs before this end-stage is reached (Group I) necrotizing arteriolitis may be entirely absent.

Malignant Hypertension

Etiology: We have been led to postulate both a toxic and a spastic factor to explain the characteristic lesions of malignant hypertension. The only exogenous toxin of known etiological significance is lead. Recent work has shown, however, that other conditions may act as precursors of malignant hypertension and lead ultimately to malignant nephrosclerosis. Such are basophil adenoma of the pituitary [27] and eclamptic toxemia of pregnancy.[28] This suggests that any hypertensive state may give rise to the malignant form of hypertension. There is considerable evidence that acute glomerulonephritis, the most common cause of hypertension in young subjects, may similarly act as a precursor of malignant hypertension. Persistent hypertension has been reported to follow acute glomerulonephritis even though the renal lesion has apparently healed (Longcope,[29] Van Slyke [30]). It might be expected that hypertension of such an origin could pass over to the malignant form. Volhard [3] has reported a case of glomerulonephritis which suggests this sequence of events (case Joh. Ei., p. 1439). The histological diagnosis of malignant nephrosclerosis was made, although from the description it is not clear whether terminally the glomerular lesion was focal or diffuse. The following case is an example of acute nephritis in which apparently the diffuse glomerular lesion healed, and which was followed later by malignant nephrosclerosis.

CASE 1. E. S., first admitted May, 1924, aged 8 years, with 2 days history of bloody urine followed by headaches and convulsions coming on after an attack of acute tonsillitis. *Urine:* Specific gravity 1010 to 1015; albumin, slight trace.

Well until second admission August, 1933. Three months pregnant.* Blood pressure 228/158. *Urine:* Specific gravity 1006; large trace of albumin; occasional white and red blood cells in sediment. Pregnancy terminated.

Third admission December, 1934. Two and a half months pregnant. Blood

* In view of the early stage of pregnancy it is unlikely that a hypertension of such severity could be attributable to a pregnancy toxemia.

pressure 230/100. *Urine:* Specific gravity 1009; large trace of albumin. Therapeutic abortion and sterilization performed.

Final admission February, 1935, complaining of increasing dyspnea and abdominal swelling. *Physical Examination:* Orthopneic, pale, signs of heart failure. Fundi not well seen. Blood pressure 270/165. *Urine:* Specific gravity 1008; large trace of albumin; granular casts and occasional red cells in sediment. Non-protein nitrogen 150 mg. per cent rising to 325. Developed pericardial friction rub and died in uremic coma.

Histological Findings in the Kidney

Multiple areas of fresh anemic infarction with a broad hemorrhagic border in the cortex.

Arteries: Severe diffuse endarteritis of large, medium and small vessels. Extensive necrotizing arteriolitis. Fibrin thrombi are present in necrotic vessels within the substance of the infarcts but are not seen outside these areas.

Glomeruli: Majority are normal. Remainder show increase in nuclei, necrosis of capillary walls, occasional adhesions, a few polymorphonuclear leukocytes present.

Tubules: Considerable tubular hyperplasia.

The conception that acute glomerulonephritis may act as a precursor of malignant hypertension is consistent with the occurrence of arteriolitis and endarteritis in the kidney in chronic glomerulonephritis. The high blood pressure which develops in the later stages of diffuse glomerulonephritis is usually moderate in degree, but cases are not infrequently encountered, especially in young subjects where the blood pressure is extremely high. In such cases hypertensive retinopathy is frequently present and the rapid downhill course of the disease is more characteristic of malignant hypertension than of chronic glomerulitis. The following is a typical example.

CASE 2. J. P., male, aged 27 years, admitted Nov. 8, 1932, with a 2 years history of "kidney trouble." Frequency day and night and hematuria. There was a history of severe headache, shortness of breath, swelling of face and misty vision for 1 week following a cold in the head.

Physical Examination: This revealed a pale, ill-looking man, orthopneic and coughing up blood-stained sputum. Retinal examination showed papilloedema with extensive exudates and hemorrhages. Blood pressure 240/140 mm. Hg.

Urine: Specific gravity maximum 1012; large trace of albumin; many red cells and leukocytes in sediment.

Blood: Non-protein nitrogen 130 mg. per 100 cc.

Patient died in uremia on day after admission.

Macroscopic Examination of Kidneys: Combined weight 160 gm. Capsule strips with difficulty from yellowish gray, coarsely granular surface. Cut surface boggy. Cortex diminished in size.

Histological Examination of Kidneys: Chronic diffuse glomerulonephritis. Every glomerulus involved. Fairly extensive necrotizing arteriolitis and productive endarteritis affecting small vessels only.

The difficulty in making a histological diagnosis in such cases arises from the simultaneous occurrence of diffuse glomerulonephritis and the arterial lesions — endarteritis and necrotizing arteriolitis. Two theories have been suggested:

(1) The arterial lesions are regarded as secondary to the glomerular inflammation. This explanation, which is maintained by Fahr, is apparently supported by the fact that the vascular lesions are confined to the kidney. If, however, the arteriolitis and endarteritis are regarded as part of a generalized vascular disease their occurrence only in this situation can be explained on the basis of the excessive vascular strain in an already damaged kidney. In pure malignant nephrosclerosis the vascular lesions may similarly be found only in the kidney. Hence the fact that arterial and arteriolar changes in glomerulonephritis are not found outside the kidney does not disprove the theory that they depend on a generalized vascular disturbance.

(2) There remains the possibility that we are dealing with coincident malignant nephrosclerosis and chronic glomerulonephritis. The frequency of this association, however, suggests a closer relation between the two conditions. It has frequently been stated that arteriolitis also occurs in acute nephritis (Löhlein,[31] Fishberg [32] and others) and the explanation has been offered that the same toxin produces both the glomerulonephritis and the arteriolitis. Such a toxin may be allergic in origin (Masugi [33]). It appears moreover that acute nephritis is not to be regarded as a purely renal disorder but rather as a general vascular disturbance which may manifest itself before the kidney shows signs of involvement. Assuming that one toxin (? allergic) may produce both the arteriolar and glomerular lesions in acute nephritis, the same explanation may hold for the lesions found in chronic glomerulonephritis. The acute stage of the disease may be regarded as resulting in a hypersensitive state of the general arterial system as well as of the glomeruli. In its progress the disease may involve either the glomeruli or the blood vessels, or both. Accordingly we may encounter any of the above mentioned sequelae of acute glomerulonephritis, *i.e.* chronic diffuse glomerulonephritis, malignant nephrosclerosis, or chronic glomerulonephritis with vascular lesions in the kidney.

Periarteritis Nodosa and Malignant Nephrosclerosis

The close similarity between these conditions in many instances led Fahr to the opinion that malignant nephrosclerosis might be regarded as a special form of periarteritis nodosa in which the arteriolar lesions were for the most part confined to the kidney — a view which agreed with his theory of the renal origin of malignant hypertension. If, however, we are correct in regarding malignant hypertension as a primary generalized vascular disturbance, and the renal vascular lesions as secondary, it is necessary to find an explanation for the association of malignant hypertension and periarteritis nodosa, especially since Volhard states that in these cases the hypertension is of the so-called "pale" type. Since the summarizing articles on periarteritis nodosa do not discuss in detail the relation of kidney involvement to hypertension, especially in its malignant form, we have made an analysis of the original case reports in the literature.

Cases with insufficient information concerning blood pressure, heart weight or kidney involvement were eliminated. Where a diagnosis of diffuse glomerulonephritis was made or where kidney involvement was definitely stated to be focal the case was also excluded. There remained some 75 cases with a diffuse distribution of the arterial lesion in the kidney — so-called "infarcted contracted kidneys." Forty-seven cases (62 per cent) showed evidence of hypertension (in 5 cases based on cardiac hypertrophy at autopsy). In 28 cases (37 per cent) a normal blood pressure was observed (in 5 cases no note was made of the blood pressure but the heart weight was normal at autopsy). Eighteen of the cases with hypertension showed a systolic pressure at or above 200 mm. Hg.

Relation of Hypertension to Kidney Involvement: We have pointed out that the majority of cases (62 per cent) with diffuse arterial lesions in the kidney showed hypertension. Where diffuse lesions in the kidney were absent no elevation of blood pressure was found. On the clinical side complete renal function tests were only occasionally available, hence the occurrence of red blood cells, albumin and casts in the urine had to be taken as clinical evidence of renal involvement. Eighty-four per cent of cases with hypertension showed such involvement but similar findings were also present in 37 per cent of cases without hypertension. Thus, as has previously

been stated, there appears to be no rigid relation between hypertension and renal involvement in periarteritis nodosa.

The Nature of the Hypertension: We usually find that the elevation of blood pressure in periarteritis nodosa is gradual in development, moderate in severity and, in cases where a full description is given, appears to be preceded by the renal involvement. This suggests that we are in fact dealing with a renal hypertension. Cases are, however, observed where extensive arterial lesions are present in the kidney with death in uremia but with no hypertension. It is possible that the diffuse involvement of the heart muscle frequently observed in periarteritis nodosa may in such cases prevent the blood pressure from rising.

An attempt to separate cases of the malignant type was made by paying special attention to the ophthalmoscopic findings. In 15 cases with hypertension where examination of the fundus occuli was reported, 7 were normal, in 6 of these [34-39] at a time when signs of renal insufficiency were already present (*i.e.* diminished concentrating power of the urine or raised non-protein nitrogen). Eight cases showed signs of retinopathy, but whether or not this could in all cases be considered of the malignant type is doubtful. The reports of these cases were, however, analyzed in an attempt to determine whether the hypertension was preceded by renal involvement. We came to the conclusion that such was not invariably the case. In 5 cases [40-44] hypertensive retinopathy and renal insufficiency were already present on the patient's admission to the hospital. In 2 cases, however, there was a history of high blood pressure preceding the periarteritis nodosa by 7 years [45] and 5 years.[46] In only 1 case did retinopathy appear while renal insufficiency was developing.[47] It may be of interest to notice that in 2 cases [48, 49] a previous history of lead poisoning is mentioned.

From the literature, therefore, it appears that the hypertension associated with periarteritis nodosa is only occasionally of the malignant type. More often it is in the nature of renal hypertension — moderate in severity, late in development, and lacking the retinal signs which characterize malignant hypertension. Malignant nephrosclerosis and periarteritis nodosa differ therefore in certain important respects. In the former, hypertension is of primary vascular origin, is malignant in type and terminates in renal failure. Histologically productive endarteritis is the most characteristic

arterial lesion and is regarded as the result of a severe prolonged vascular spasm. Arteriolitis on the other hand appears to be a terminal manifestation and is related more closely to renal insufficiency than to hypertension.

In periarteritis nodosa inflammatory lesions of the vessels occur as the primary event and, as in malignant nephrosclerosis, appear to be more closely related to some toxic factor than to hypertension. In fact hypertension only arises in the majority of cases when destruction of the kidneys by vascular changes has resulted in renal insufficiency. The inflammatory lesions in the vessels predominate and endarteritis, as Volhard points out, is irregular in distribution. Hypertension of the malignant type occurs in relatively few instances and in these some additional factor appears to be present; thus antecedent hypertension was present in 2 cases in the literature and in 2 others a history of lead poisoning was obtained.

We are led to the conclusion that the etiological agent (allergic or otherwise) which produces the inflammatory lesions in the arteries in periarteritis nodosa is not *per se* responsible for the malignant character of the hypertension but only when acting on arteries which show an abnormal reactivity. We were previously led to postulate a difference in reactivity of the arteries to explain the different character of malignant hypertension in young and old subjects; and we suggested above that the blood vessels might be "sensitized" by an attack of acute diffuse glomerulonephritis. The whole evidence leads us to the conclusion that two factors are necessary for the development of malignant nephrosclerosis — a preëxisting hyperactivity or "sensitization" of the arteries on which is superimposed some precipitating factor, allergic or otherwise.

Summary and Conclusions

(*A*) Benign hypertension and benign nephrosclerosis may show a parallel development but in the early stages are not causally related. In the later stages, however, there may be a reciprocal relationship, *i.e.*:

(1) Hypertension acts as an accelerating factor on the development of arteriosclerosis.

(2) Arterial and arteriolar sclerosis of the kidney, when severe enough to produce impairment of renal function, may give rise to

"renal" fixation of the hypertension. Such cases are termed "decompensated benign nephrosclerosis" since clinical and histological evidence shows that the impairment of function is of true renal origin.

(*B*) Malignant hypertension and malignant nephrosclerosis, on the other hand, show a definite correlation.

(1) On clinical and histological grounds malignant hypertension is to be regarded as a primary generalized vascular disease of which malignant nephrosclerosis represents the "renal end-stage." Cases are described in which death occurs from malignant hypertension before the renal end-stage is reached.

(2) When malignant hypertension progresses to the stage of malignant nephrosclerosis, the condition is clinically and histologically characteristic, as described by Volhard and Fahr. The main objection to their classification is the existence of so-called "borderline" cases, which are neither clinically nor histologically characteristic. Of these cases, in our interpretation, one group consists of cases of malignant hypertension in which death occurs before the renal phase develops, the other group comprises older subjects in whom the malignant hypertension is less fulminant and may be superimposed on benign nephrosclerosis.

(3) Endarteritis in its diffuse form is regarded as the most characteristic histological sign of malignant hypertension. Arteriolitis (arteriolar necrosis) is more closely related to the terminal renal failure than to the hypertension itself.

(4) Various hypertensive states may act as precursors of malignant hypertension. Evidence is presented that diffuse glomerulonephritis may similarly be associated with or followed by malignant hypertension, thereby explaining the occurrence of the "specific" vascular lesions in the kidney in diffuse glomerulonephritis.

(5) A study of the relation of periarteritis nodosa to malignant nephrosclerosis provides suggestive evidence that two factors are necessary for the development of malignant hypertension, namely, a preëxisting hyperactivity or "sensitivity" of the arteries, on which is superimposed a precipitating factor, allergic or otherwise.

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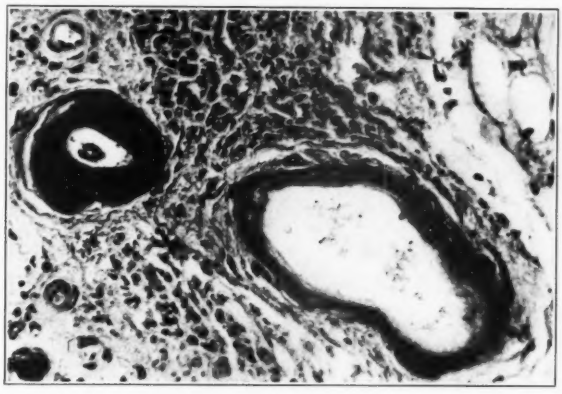
DESCRIPTION OF PLATES

PLATE 7

FIG. 1. Fibrinoid degeneration in the small vessel staining dark purplish blue with eosin-methylene blue. Wall of large vessel shows hyalinization only. Stains red.

FIG. 2. Similar fibrinoid degeneration showing irregular, flake-like appearance of fibrinoid material in hyaline mass. Eosin-methylene blue.

FIG. 3. Early alterative glomerulitis showing necrosis of swollen epithelial cells with adhesion to Bowman's capsule at this point. Eosin-methylene blue.



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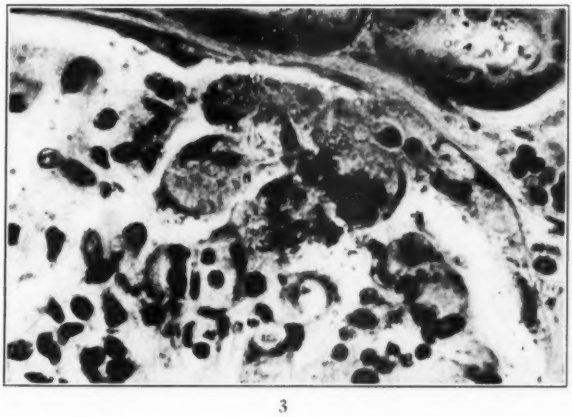

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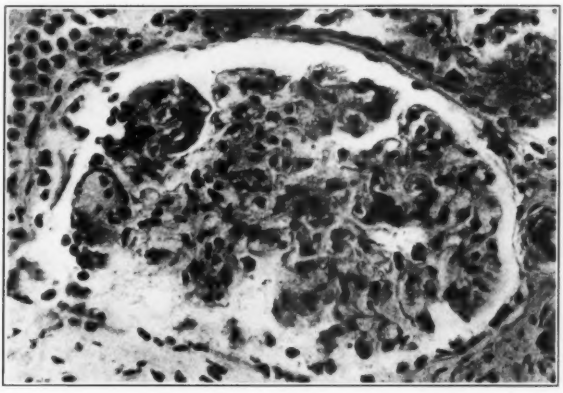
Plate 8

Fig. 4. Early alterative glomerulitis; several loops show necrotizing processes. There seems to be a slight increase of nuclei in these areas. Eosin-methylene blue.

Fig. 5. Alterative glomerulitis showing acute necrosis at one point (dark area which stains purplish). Several old adhesions. Eosin-methylene blue.

Fig. 6. Extensive necrotizing glomerulitis; several loops involved simultaneously. Eosin-methylene blue.



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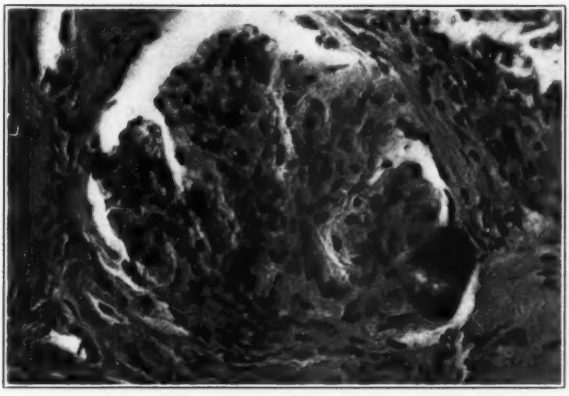

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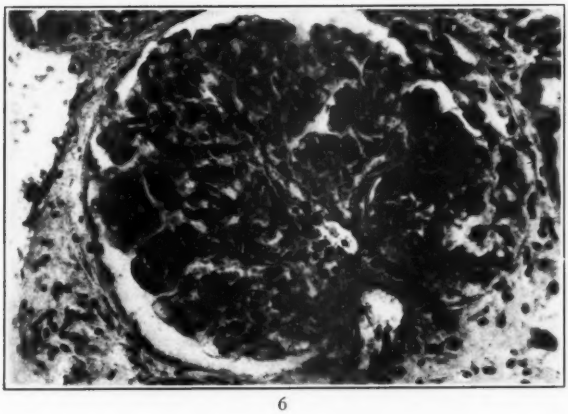

6

INTERCAPILLARY LESIONS IN THE GLOMERULI OF THE KIDNEY *

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INTRODUCTION

Since the existence of an intercapillary connective tissue, not only confined to the hilum but also extending to the periphery, has been definitely recognized in the normal glomerulus,[1, 2, 3] more attention has been paid to the pathological changes in this connective tissue framework. MacCallum, in particular,[4] has recently analyzed the changes in the intercapillary connective tissue which are associated with various pathological conditions in the kidney. He describes edema, amyloid degeneration, hyalinization and growth of the connective tissue. Of special interest in relation to the material we present is his statement that intracapillary glomerulonephritis consists in an increase of intercapillary connective tissue. For this reason he suggests the term "intercapillary" in place of "intracapillary" glomerulonephritis. From his description it is not quite clear whether he restricts the term "intracapillary glomerulonephritis" to the cases so-called by Fahr [5] or includes also the common "extracapillary glomerulonephritis."

One of us [6] has recently described the frequently observed broadening of the connective tissue of the glomerulus as an aging process which apparently develops independently of hypertension and arteriosclerosis. Further studies show that in a certain group of cases this change may so dominate the histological picture as to give a characteristic appearance. Since the clinical findings may be equally characteristic, it seems justifiable to describe these cases as a special group. In attempting to do so, however, considerable difficulties are encountered, especially in advanced cases, in differentiating them from so-called intracapillary glomerulonephritis (Fahr). We have, therefore, contrasted the features of this special group with those of

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true intracapillary and extracapillary glomerulonephritis. A detailed study of the basement membrane enables us to make a definite distinction in the majority of cases.

Staining Method

1. Lithium carmine 1½ hours at 55° C.
   (Stain contains 2½ gm. carmine in 100 cc. saturated lithium carbonate.)
2. Without contact with water transfer to 1 per cent hydrochloric acid in 70 per cent alcohol for 3 minutes. Change once.
3. Wash with distilled water.
4. 1 per cent phosphomolybdic acid 30 seconds.
5. Wash with distilled water once.
6. Aniline blue-orange G stain ¾ hour at 55° C.
   (0.5 gm. aniline blue with 2 gm. orange G in 100 cc. 1 per cent phosphomolybdic acid. Stir well and allow to stand a few hours. Filter.)
7. Wash with distilled water several times.
8. 1 per cent phosphomolybdic acid 45 seconds.
9. Wash with distilled water once.
10. Differentiate in 95 per cent alcohol to which is added 1 cc. 15 per cent sodium hydroxide per 100 cc. until first traces of reddish stain appear.
11. Wash quickly in excess of 95 per cent alcohol. The stain washed out turns blue. Continue until no further blue appears.
12. Absolute alcohol, xylol, balsam.

Fresh Zenker-fixed material must be used. Paraffin embedding and thin sections are necessary. If the section is not differentiated enough it may be brought back to alkaline alcohol for a very short time.

Results: Connective tissue and basement membranes deep blue, nuclei red with clear structure. Cytoplasm grayish pink, hyaline droplets blue. Red blood cells golden yellow or occasionally greenish. The colors are more delicate than in the Heidenhain stain and details of the cytoplasm and basement membrane are clearly brought out.

A. Intercapillary Glomerulosclerosis

The term intercapillary glomerulosclerosis has been applied to the group of cases under discussion, of which we present 8 examples showing different stages of the glomerular lesion.

Case 1. B. P., female, aged 62 years, on admission complained of shortness of breath and drowsiness. There was a 3 years history of diabetes with 2 months known hypertension.

Physical Examination: The patient was semicomatose with signs of cardiac failure, and generalized edema including arms, face and neck was present. Blood pressure 180/100 mm. Hg.

Urine: Specific gravity 1004–1010; large trace of albumin; many leukocytes in sediment; sugar negative.

Blood: Non-protein nitrogen 58 mg. per 100 cc.

Autopsy Findings

Anatomical Diagnosis: Myocardial failure.

Gross Appearance of Kidneys: Combined weight 300 gm. Average in size. The capsule strips with difficulty from a granular reddish brown surface with occasional stellate scars. On section the cortex averages 5 mm. in thickness, is reddish brown with normal striations and clearly demarcated from the medulla.

Case 2. H. G., male, aged 60 years, was admitted in extremis complaining of pain in the chest and cough. No previous history was obtainable.

Physical Examination: Generalized edema including the hands and face was present. Blood pressure 160/70 mm. Hg. Signs of bronchopneumonia and cardiac failure were present and the patient died 3 hours after admission.

Autopsy Findings

Anatomical Diagnoses: Chronic nephritis, bronchopneumonia.

Gross Appearance of Kidneys: Combined weight 380 gm. The capsule strips with ease, leaving a finely granular surface. On section the cortex is pale grayish purple, 5 mm. in thickness, with grayish white medulla.

Case 3. A. T., female, aged 63 years, was admitted with a complaint of gradually increasing edema for 10 months with shortness of breath on exertion. There was a previous history of diabetes.

Physical Examination: Edema of legs, thighs, abdomen, arms and face was present. Blood pressure 120/80 mm. Hg.

Urine: Specific gravity 1017–1024; large trace of albumin; sugar ++.

Autopsy Findings

Anatomical Diagnoses: Myocardial failure, arteriosclerosis and diabetic nephrosis.

Gross Appearance of Kidneys: Combined weight 240 gm. The capsule strips with difficulty from a grayish brown, very finely nodular surface containing several smooth depressed scars. Cut surface reveals a cortex 3–5 mm. broad, grayish brown in color, and clearly demarcated from darker red medulla.

CASE 4. M. H., female, aged 51 years, was admitted in a semicomatose condition with twitching of muscles, vomiting and shortness of breath. There was a 10 years history of diabetes.

Physical Examination: Edema of ankles, bilateral cataract and severe arteriosclerosis were present. Blood pressure 190/80 mm. Hg.

Urine: Specific gravity 1012; large trace of albumin; many leukocytes and occasional red cells in sediment.

Blood: Non-protein nitrogen 170 mg. per 100 cc.

Autopsy Findings

Anatomical Diagnoses: Generalized arteriosclerosis, bronchopneumonia.

Gross Appearance of Kidneys: Combined weight 230 gm. The capsule strips easily leaving a yellowish gray, coarsely granular surface. On section a very pale grayish yellow, finely granular cortex 5 mm. thick, with pale grayish red medulla is seen. Marked atheroma and calcification of renal vessels is present.

CASE 5. L. S., female, aged 48 years, was admitted with a complaint of swelling of legs and blurring of vision. There was a 3 years history of diabetes.

Physical Examination: The patient was stuporous with Cheyne-Stokes respiration and generalized edema was present. There were retinal exudates and hemorrhages. Blood pressure 230/110 mm. Hg.

Urine: Large trace of albumin; sugar +.

Blood: Non-protein nitrogen 133 mg. per 100 cc.; blood sugar 275 mg. per cent.

Autopsy Findings

Anatomical Diagnoses: Generalized anasarca, myocardial failure.

Gross Appearance of Kidneys: Combined weight 370 gm. The surface is granular. On section the cortex is 6 mm. thick, and yellowish gray-white in color.

CASE 6. A. H., female, aged 49 years, was admitted with a 2 weeks history of vomiting and oliguria. There was a 15 years history of diabetes. Right nephrectomy for renal calculi was performed 18 years before.

Physical Examination: The patient was comatose with Cheyne-Stokes respiration.

Urine: Albumin present; many leukocytes in sediment; sugar ++.

Blood: Non-protein nitrogen 150 mg. per 100 cc.

Autopsy Findings

Anatomical Diagnoses: Generalized arteriosclerosis, chronic nephritis.

Gross Appearance of Kidney: Weight 280 gm. (left). The capsule strips with difficulty leaving a coarsely granular surface. On section the cortex is grayish red with multiple irregular scars scattered throughout and poorly demarcated from the medulla.

CASE 7. C. H., male, aged 68 years, was admitted complaining of dyspnea on exertion and edema of feet. There was a 6 years history of diabetes.

Physical Examination: Edema of ankles was present. Blood pressure 230/110 mm. Hg.

Urine: Specific gravity 1010–1022; large trace of albumin; sugar +.
Blood: Non-protein nitrogen 25 mg. per 100 cc.

Autopsy Findings

Anatomical Diagnoses: Hypernephroma, pulmonary infarction.
Gross Appearance of Kidneys: The right kidney weighs 375 gm. The capsule strips easily leaving a smooth, pale, swollen surface. On section the cortex is 7 mm. broad and appears pale and swollen. The left kidney contains a hypernephroma.

CASE 8. A. F., male, aged 58 years, was admitted complaining of increasing swelling of the feet, legs and abdomen. There was a 5 years history of diabetes.
Physical Examination: Bilateral cataract present. Pitting edema of legs and abdomen and marked ascites. Blood pressure 190/100 mm. Hg.
Urine: Specific gravity 1007–1015; large trace of albumin; many leukocytes and red cells in sediment.
Blood: Non-protein nitrogen 87 mg. per 100 cc.

Autopsy Findings

Anatomical Diagnoses: Generalized atheroma, myocardial failure.
Gross Appearance of Kidneys: Combined weight 400 gm. The capsule strips with difficulty from a finely granular surface. The cut surface is mottled gray and yellow. The cortex is 6 mm. broad, well demarcated from the medulla.

Microscopic Appearances in the Kidney

(1) *The Glomeruli:* The most striking feature is the great regularity with which the hyalinization of the glomerulus is confined to its center, or even to the center of one lobule (Fig. 1). With the eosin-methylene blue stain an entirely homogeneous mass is seen, suggestive of amyloid, but negative reactions are obtained with all the amyloid stains. Fat stains (Sudan III) usually give a homogeneous pinkish color, while double refraction is only exceptionally present in smaller circumscribed areas. Toward the periphery of the glomerulus the basement membrane of the capillaries seems to emerge from the hyaline mass but its outline is sharply defined and usually surrounds a widely patent lumen (Fig. 2). The basement membrane may be delicate like the normal one, or somewhat thickened, but is never wrinkled or split (Fig. 3). The number of capillaries is apparently reduced — in many cases there remains only a ring of open capillaries surrounding the central hyaline mass (Fig. 1). The remainder appear to have been buried in it and to have disappeared.

The hyaline mass itself obviously represents a broadening of the intercapillary connective tissue. This can be observed particularly

well at the hilum. A very high degree of arteriosclerosis with fatty degeneration of the arterioles is present in most of the cases and the hyaline material is seen to be continuous from the vasa afferentia into the intraglomerular mass as it extends from the center of the lobules to the periphery (Fig. 4). The intercapillary hyalinization is not, however, to be regarded merely as an extension of the degenerative process from the vas afferens since it is found in glomeruli where the vas afferens is normal. Not infrequently pictures are encountered similar to those recently described by one of us as an "aging process of the glomerulus," but the axial thickening is much more massive and striking. It must be emphasized, however, that there is only a difference in degree between the less marked changes frequently observed in senile kidneys and the lesion here described. Although the basement membrane of the capillaries seems to be well preserved for a long time, the change gradually extends to the periphery. This mode of extension is most characteristic. Eventually the capillary walls thicken homogeneously and near the central hyaline mass they collapse and finally merge with the central hyalin (Fig. 5). It is also possible, as MacCallum points out, that the capillaries are partly pushed toward the periphery and preserved there for a long time.

While the capillaries are fusing with the central hyalin, the nuclei, especially those of the endothelial cells, appear well preserved and are crowded together (Fig. 6). This process may be observed step by step: the lumen of the capillaries becomes a narrow slit, the endothelial nuclei increase in density but retain their elongated form and are finally entirely embedded in a homogeneous hyaline mass (Fig.7). This may give rise to an apparent increase in nuclei which simulates an insidious proliferative process, thereby leading to misinterpretations, as we shall see later. The nuclei are often arranged in "onion layers" at the periphery of the hyalin (Fig. 6), presumably originating from the endothelial cells of the collapsed capillary loops. Two reasons prevent one from attributing this appearance to true proliferation: (1) Immature nuclei and mitoses are never seen — on the contrary the nuclei are almost invariably pyknotic and in a state of necrobiosis; (2) The mode of development of the apparent increase in nuclei can be seen to be the result of a regressive process. There is, furthermore, no definite proof of an inflammatory process. Regarding the origin of the nuclei many observations have led to the con-

viction that sometimes at least they originate from the endothelial cells of collapsed capillaries. Whether in addition there is a true "growth" of interstitial tissue as MacCallum assumes, it is difficult to decide. The more centrally situated nuclei lie in a dense homogeneous mass which certainly originates from the intercapillary connective tissue, but in later stages the capillaries with their endothelial and epithelial cells become embedded, and the origin of the nuclei cannot be recognized.

(2) *Capsular Changes:* Severe changes also occur in the glomerular capsule. A substance is deposited which at first appears translucent with a slightly pinkish stain, and later becomes more homogeneous, hyalin-like, and often contains abundant lipoid. The mass lies between the basement membrane and the epithelial layer of Bowman's capsule lifting the epithelial cells (Fig. 8). It may be deposited in such quantity that the capsular space is greatly narrowed (Fig. 9). A connective tissue reaction appears in the outer layers in later stages only — apparently a resorptive or organizing process. Broadening of the connective tissue occurs by the formation of concentric layers of fibrils and nuclei. The mode of development of this capsular change, which frequently accompanies the glomerular lesion, definitely indicates the primary degenerative character of the whole process.

The intercapillary process described above is practically diffuse, in the sense that almost all the glomeruli are affected. Although the severity of the lesion may vary, in a few cases it is the only lesion which can be found. In others, however, a scanty focal glomerulitis is present of the type we have described in decompensated benign nephrosclerosis and malignant nephrosclerosis. There are, in these instances, as in decompensated benign nephrosclerosis, definite signs histologically and clinically of renal insufficiency and we interpret these cases as decompensated benign or malignant nephrosclerosis complicated by intercapillary glomerulosclerosis.

The tubular changes have no special significance. The degenerative processes common to all arteriosclerotic processes are found. There is, however, in most cases a striking deposition of fat and doubly refracting lipoid in the tubules and in the interstitial tissue. No diagnostic significance, however, is attached to lipoid deposition in our cases since the amount was never sufficient to give the gross appearance of a lipoid nephrosis. Moreover, many cases of severe

uncomplicated arteriosclerosis of the kidneys show a fairly high degree of lipoid infiltration of the tubules and interstitial tissue. One case grossly appeared to resemble a nephrosis but lipoid deposition was not extraordinary, the tubules showing chiefly albuminuric and hyaline droplet degeneration.

Comment

The Clinical Picture: The separation of the above group of cases, especially from intracapillary glomerulonephritis, has been made on histological grounds which are discussed later. On reviewing the clinical data it was surprising to find a previous history of diabetes in all these cases, with the exception of one (Case 2) in which death occurred 3 hours after admission and no history was obtainable.* It must be emphasized that only a small proportion of cases of diabetes appears to show this lesion at autopsy. A second striking feature was severe and widespread edema. The clinical picture appears in fact to be almost as characteristic as the histological one: the patients are relatively old; hypertension is present, usually of the benign type, and the kidneys frequently show signs of decompensation; there is a history of diabetes usually of long standing; the presenting symptoms may be those of edema of the nephrotic type, renal decompensation or heart failure; the urine contains large amounts of albumin and there is usually impairment of concentrating power with or without nitrogen retention.

Although some degree of cardiac failure is frequently present, the edema is out of all proportion to this and may be extreme when no signs of heart failure can be demonstrated. Furthermore, its generalized distribution, especially its extension to the arms and face, leads us to regard it as nephrotic rather than cardiac in type, and this conclusion is supported by the constant finding of severe albuminuria.

The Pathological Picture: The gross appearance of the kidneys is not characteristic. They present the picture of arteriosclerotic contraction which may be in part or completely obscured by the signs of

* Since the investigation was completed several other instances of intercapillary glomerulosclerosis have been encountered. All were cases of diabetes except one in which a questionable reducing reaction was present in the urine, but no information was available in regard to the previous history.

nephrosis, *i.e.* they may be enlarged and swollen with grayish or yellowish external and cut surfaces.

The histological picture is, however, very characteristic. Arteriosclerosis is present, usually of very high degree, fatty degeneration of the arterioles being unusually conspicuous. Intercapillary hyaline change is discernible in most of the glomeruli even under low power. The special stain is necessary to demonstrate the earlier stages of the process but when this is used the lesion cannot be overlooked. All degrees of this hyaline change can be observed down to those produced simply by the aging process, and the cases described above represent an extremely severe type.

The tubules very often show fatty degeneration, and lipoid is frequently found in the interstitial tissue.

In addition to these characteristic features the signs of true renal decompensation we have previously described are commonly found, and when this is the case the capsular adhesions though focal in distribution may be surprisingly frequent.

B. Intracapillary Glomerulonephritis (Fahr)

We have referred to the great difficulties which may arise in differentiating late stages of intercapillary glomerulosclerosis and so-called intracapillary glomerulonephritis. The latter differs from the common extracapillary glomerulonephritis in the absence or very scanty occurrence of capsular proliferation. In the subchronic stage, intracapillary glomerulonephritis is frequently complicated by the "nephrotische Einschlag." Histologically a peculiar hyalinization of the glomerulus occurs which accentuates its lobulation in the same way as in intercapillary glomerulosclerosis (Fig. 10). In the early stages abundant leukocytes appear in the capillaries and emigrate into the capsular space and convoluted tubules. In the later stages the only finding may be an increase in nuclei which Fahr interprets as an insidious endothelial proliferation. He believes that hyalinization is due to fusion of the thickened capillary walls. Adhesions are frequently found and thickening of the connective tissue of Bowman's capsule is often present. During the earlier stages there is little difficulty in diagnosing the inflammatory nature of the disease on account of the presence of leukocytic infiltration. In the chronic stages, however, where leukocytic infiltration is almost absent, capsular ad-

hesions may occur only focally and a definite decision may be very difficult. True endothelial proliferation — or at least increase in endothelial nuclei — can sometimes be recognized, but such a finding is inconstant; in general the apparent increase in nuclei cannot be regarded as proof of an inflammatory process. Pyknotic nuclei are embedded in a hyaline mass and it may be impossible to say whether they originate from endothelial or connective tissue cells and whether there is an actual increase in nuclei or merely a crowding effect. The clinical evidence is frequently negative since the acute stage of the glomerulonephritis may pass unnoticed. Intercapillary glomerulosclerosis may, in fact, so resemble intracapillary glomerulonephritis in the late stages that a distinction is impossible. We encountered one such case.

Detailed examination of cases of intracapillary glomerulonephritis shows that in most glomeruli the main mass of hyalin is localized at the center of the lobules; and as in intercapillary glomerulosclerosis a ring of open capillaries is left at the periphery. Although red blood cells are frequently seen in these peripheral capillaries, the lumen is rather narrow and never distended. The hyalinization is easily distinguished from that which occurs in the more common extracapillary glomerulonephritis. In this condition there is irregular thickening of the capillary loops and the lumen is narrowed by the broadening and splitting of the basement membrane. The process often affects the glomerulus focally leaving the unaffected parts free. Moreover, the hyalinization tends to start in the periphery, whereas in intracapillary glomerulonephritis it begins at the center of the lobule.

The central origin of hyalinization in intracapillary glomerulonephritis with preservation of the peripheral capillaries indicates that the degeneration of the connective tissue framework occurs independently of and is superimposed on hyalinization of the capillary wall. If the latter alone were present we should expect the hyalinization of the glomerulus to be diffusely distributed, since in the earliest stages of the disease all the capillaries are involved. The following observation gives more definite proof that hyalinization of the intercapillary tissue occurs as an independent process. In some cases one encounters occasional glomeruli which are not involved in the otherwise diffuse inflammatory lesion and which show hyalinization of the intercapillary connective tissue framework without any change in the peripheral zone. In some of these instances a marked swelling

and hyaline droplet degeneration of epithelial cells is present but in others the glomerulus is normal except for the change in the intercapillary tissue (Fig. 11).

It has already been emphasized that the apparent increase in nuclei in the hyaline mass in intracapillary glomerulonephritis may be attributed to crowding of the endothelial nuclei of collapsed capillaries and does not furnish proof of an inflammatory process. Changes occur, however, in the basement membrane of the preserved peripheral capillaries which in our experience are characteristic of such a process. These are blurring of outline and splitting of the basement membrane, which are also commonly found in extracapillary glomerulonephritis (Fig. 12). The development of intracapillary hyaline fibers, described by McGregor, is rare in the intracapillary form.

Diffuse involvement of the capillaries by these changes definitely indicates a primary diffuse lesion of the glomerular capillaries in contradistinction to intercapillary glomerulosclerosis, even though no actual inflammatory infiltration may be demonstrated. A marked swelling of epithelial cells, which is found even in the later stages, further indicates the inflammatory nature of the process and when diffusely distributed may be regarded as a diagnostic sign. The same change is found in intercapillary glomerulosclerosis but is focal in distribution.

The capsular changes closely resemble those of intercapillary glomerulosclerosis. The same formation of connective tissue layers is present without, however, any epithelial proliferation (crescent formation). Volhard [7] considers this as a reaction to waste products which diffuse outwards from the capsular space. Fahr believes the process to be inflammatory, in keeping with the "insidious endothelial proliferation" in the glomerulus. One can, however, only state that the same capsular change occurs in association with a purely degenerative process in intercapillary glomerulosclerosis, and hence does not necessarily indicate an insidious inflammatory reaction.

Comment

In intracapillary glomerulonephritis capsular proliferation (crescent formation) is inconspicuous, but a most characteristic feature is the massive degeneration of the connective tissue framework of the glomerulus which complicates the capillary lesion. Blurring and

splitting of the basement membrane are regarded as indicative of the inflammatory nature of the process even in the late stages when cellular infiltration and proliferation are absent, and this capillary lesion differentiates the condition from intercapillary glomerulosclerosis. It is noteworthy that in cases of both arteriosclerosis and intracapillary glomerulonephritis the intercapillary degeneration tends to be accompanied by fatty tubular nephrosis and interstitial deposition of doubly refracting lipoid.

C. Extracapillary Glomerulonephritis

The changes in the basement membrane of the peripheral capillaries in intracapillary glomerulonephritis are also found in the extracapillary form. Our observations are based on McGregor's [8] detailed description of structural changes in the glomerulus in glomerulonephritis which are brought out by combined nuclear and basement membrane stains. She claims that a most characteristic sign is the development of intracapillary hyaline fibers, either from fibrin threads or from the basement membrane itself. Hyalinization of the glomerulus is assumed to result from thickening and fusion of these fibers. Our observations essentially confirm these findings with the following additions:

(1) Certain changes in the basement membrane are found in inflammatory lesions of the glomeruli and are no less characteristic than intracapillary hyaline fibers. They may in fact be present in the absence of the latter. Such changes include splitting of the basement membrane which appears blurred and definitely thickened, though not wrinkled. The membrane appears as if teased out into a meshwork of delicate fibers which narrow the lumen. Where the fibers are thick and the section cuts a loop tangentially, they give the impression of being intracapillary. It cannot be stated whether or not all the intracapillary fibers are to be explained in this way; some may well be actually split off the basement membrane. In any case the distribution of both intracapillary fibers and splitting of the basement membrane is irregular throughout the glomerulus. It may be diffuse or confined to small areas, but the peripheral loops are as severely involved as the more central ones. MacCallum states that he has not observed intracapillary occlusions in "intracapillary glomerulonephritis." This holds true for intracapillary glomerulone-

phritis in Fahr's sense, but in the common (extracapillary) form our observations support McGregor's contention that such occlusions are of frequent occurrence.

(2) In extracapillary glomerulonephritis there is frequently, in addition to the above changes, thickening of the intercapillary connective tissue. The points of difference from the similar lesion in intracapillary glomerulonephritis have been outlined above. In particular, the thickening is irregular and focal in its distribution throughout the glomerulus, and does not appear to have any characteristic significance.

Comment

In the common or extracapillary glomerulonephritis thickening of the intercapillary connective tissue is irregular or focal in distribution and is relatively insignificant. Intracapillary fibers, which are found in all inflammatory lesions of the glomerulus, are associated with an equally characteristic change, namely, broadening and splitting of the capillary basement membrane.

Discussion

The glomerular changes in glomerulonephritis include two characteristic elements: (1) alteration of the basement membrane leading to "intracapillary" fibrillation; and (2) a purely degenerative process, the deposition of intercapillary hyaline material. This intercapillary glomerulosclerosis is apparently an independent lesion since it may be superimposed on pure arteriosclerosis as well as on inflammatory changes in the glomeruli. It may therefore be considered as a "complication" of glomerulonephritis.

It has been shown that intercapillary glomerulosclerosis and intracapillary glomerulonephritis have in common a degenerative process in the intercapillary connective tissue. They present also a striking clinical similarity in their frequent association with the nephrotic type of edema — the "nephrotische Einschlag," which has been attributed to a general metabolic disorder. The invariable finding of diabetes in cases of pure intercapillary glomerulosclerosis lends support to such a theory.

Summary and Conclusions

Cases are described which show a striking hyaline thickening of the intercapillary connective tissue of the glomerulus. Evidence is presented which indicates that the change is degenerative in nature and suggests that arteriosclerosis and diabetes may play a part in its causation. The lesion is therefore termed intercapillary glomerulosclerosis. The characteristic clinical features are a previous history of diabetes, severe and widespread edema of the nephrotic type and gross albuminuria. Hypertension is frequently present, in many cases associated with renal decompensation.

The same histological picture frequently complicates intracapillary glomerulonephritis but in the later stages this condition is differentiated histologically by blurring and splitting of the capillary basement membrane.

In extracapillary glomerulonephritis thickening of the intercapillary connective tissue is relatively insignificant and the basement membrane changes are more pronounced.

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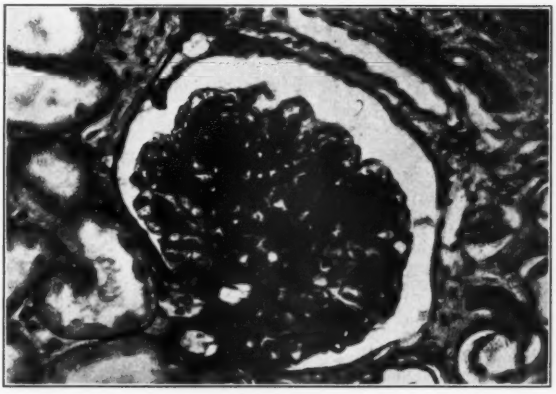
DESCRIPTION OF PLATES

PLATE 9

FIG. 1. Intercapillary glomerulosclerosis. Central hyalinization of all glomerular loops. Peripheral capillaries patent. Special basement membrane stain.

FIG. 2. Central hyalinization of peripheral loop. Capillaries wide open and contain red blood cells. Basement membrane clearly delineated and delicate. Special basement membrane stain. High power.

FIG. 3. Intercapillary hyalinization. Peripheral capillaries patent, nuclei of endothelial and epithelial cells clearly recognizable. Capillary basement membrane somewhat thickened. Special basement membrane stain. High power.



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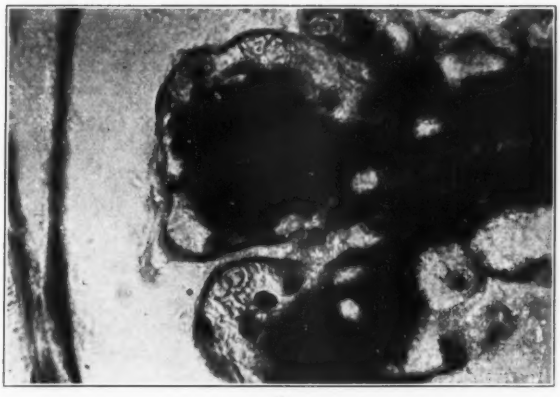

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Kimmelstiel and Wilson Intercapillary Lesions in Glomeruli of Kidney

PLATE 10

FIG. 4. Hyalinization of intercapillary connective tissue extending into most of the loops, in direct continuity with the hyaline material of the vas afferens. Special basement membrane stain. Medium power.

FIG. 5. Intercapillary hyalinization in several loops; the hyaline material encroaches upon the capillary wall which is homogeneously thickened. The capillaries are collapsed and their lumen reduced to a narrow slit. Special basement membrane stain. Medium power.

FIG. 6. Central hyalinization clearly seen even with eosin-methylene blue stain. Crowding of endothelial nuclei around collapsed capillaries gives appearance of "onion layers." Eosin-methylene blue stain. High power.

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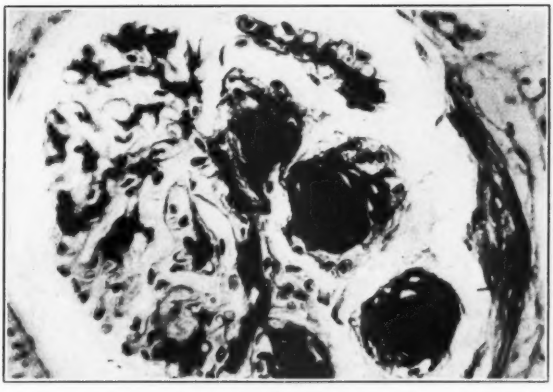

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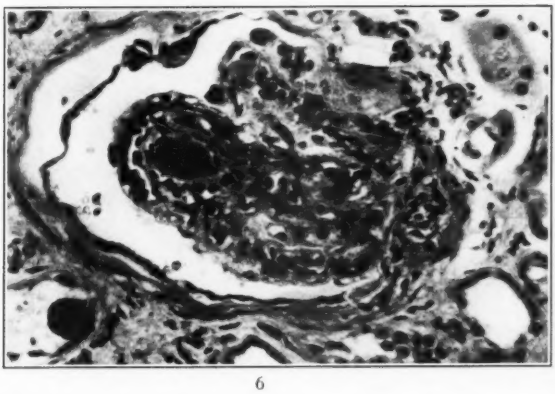

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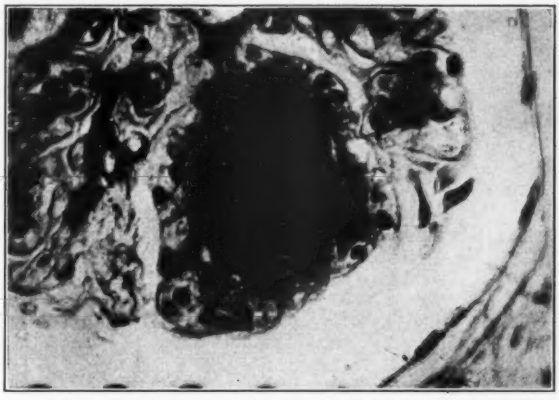
Plate 11

Fig. 7. Well preserved endothelial nuclei are seen embedded in central hyaline mass. Special basement membrane stain.

Fig. 8. Hyaline fatty mass seen between basement membrane and epithelial cells of Bowman's capsule. Special basement membrane stain. High power.

Fig. 9. Sudan III fat stain shows large fatty mass between epithelial cells and basement membrane of Bowman's capsule. The picture also shows fat in the vas afferens, some fatty degeneration of capillary loops and fat in the tubular epithelial cells. Sudan III stain. High power.



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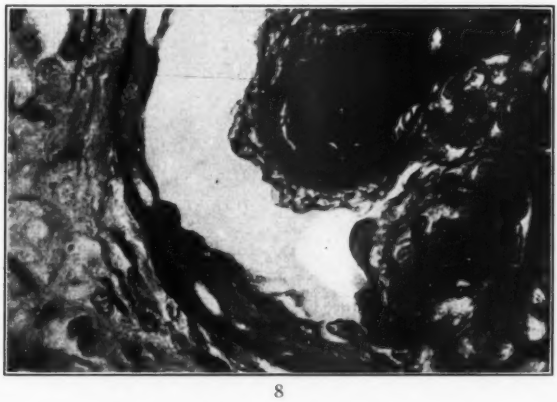

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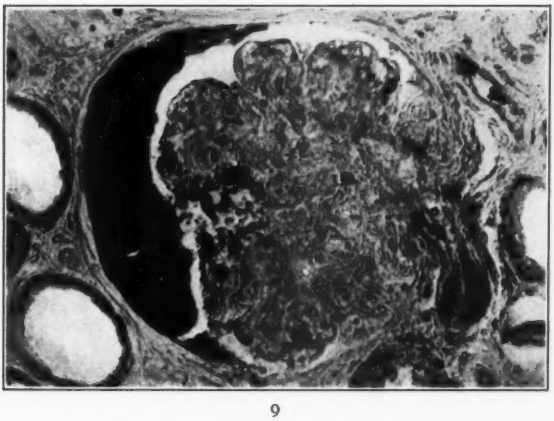

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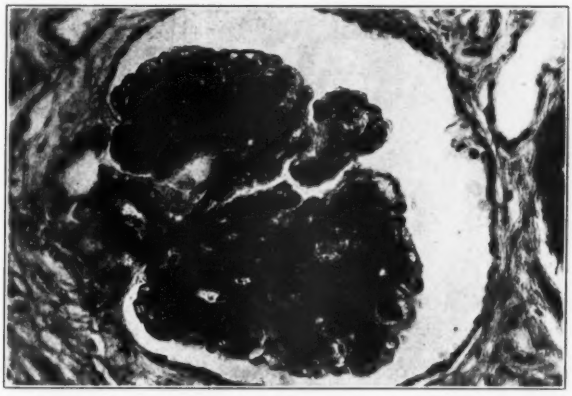
Plate 12

Fig. 10. Intracapillary glomerulonephritis. Central hyalinization identical in situation with that of intercapillary glomerulosclerosis (see Fig. 1). Notice open capillaries in the periphery. Special basement membrane stain. High power.

Fig. 11. Single glomerulus in an otherwise diffuse intracapillary glomerulonephritis. There is no other change but a severe central intercapillary hyalinization. Special basement membrane stain. High power.

Fig. 12. Intracapillary glomerulonephritis showing blurred outline of peripheral capillaries. Special basement membrane stain. High power.



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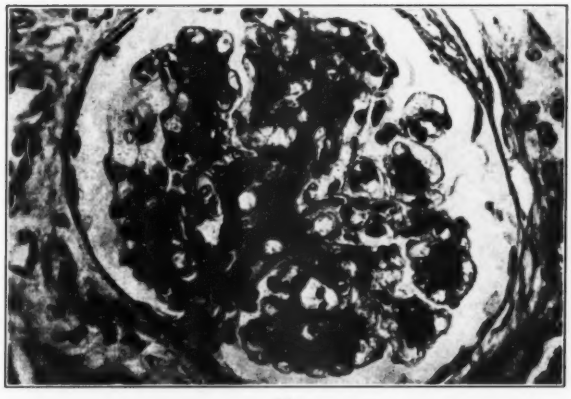

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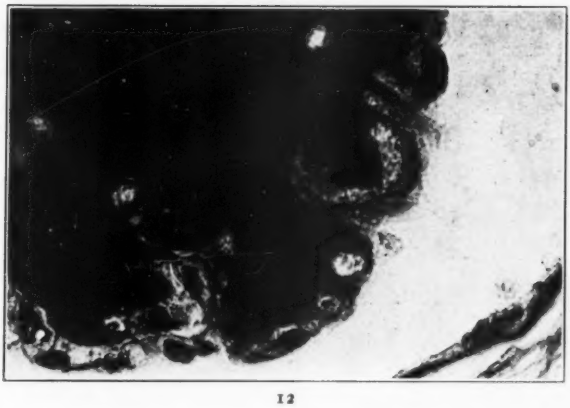

12

Kimmelstiel and Wilson Intercapillary Lesions in Glomeruli of Kidney

INFLAMMATORY LESIONS IN THE GLOMERULI IN PYELONEPHRITIS IN RELATION TO HYPERTENSION AND RENAL INSUFFICIENCY *

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Inflammatory lesions in the glomeruli in cases of pyelonephritis are relatively uncommon even when contraction of the kidney is extreme. When they occur, moreover, they are focal in distribution. In a previous communication [1] we have described in detail inflammatory changes in the glomeruli in "essential" hypertension. Such glomerulitis is also focal in distribution and is encountered in cases which have progressed to the stage of renal insufficiency, *i.e.* in malignant nephrosclerosis and in benign nephrosclerosis with "renal decompensation." We came to the conclusion that glomerulitis in these conditions is the result of combined toxic and ischemic factors. It therefore seemed of importance to study the relation of focal glomerulitis to hypertension and renal insufficiency in a disease where the renal damage results from changes in the tubules and interstitial tissue independent of the vascular elements of the kidney.

LITERATURE

Reports in the literature on the glomerular lesions in pyelonephritis are scanty. Putschar in Henke and Lubarsch's Handbook (p. 430) says "the glomeruli appear in the acute stages (of pyelonephritis) to be practically uninvolved." Russell,[2] however, describes in some detail glomerular changes in "ascending" and "interstitial" nephritis. In the former invasion of the capsular space and of the glomerular tuft by neutrophil leukocytes and desquamation of the capsular epithelium were observed in the early stages. In later stages adhesive glomerulitis and slight proliferative glomerulitis were found, accompanied in still more chronic cases by focal necroses in the tufts. In "interstitial" nephritis two main types of glomerular change were observed in the chronic stages; first, focal necroses and adhesions resembling those seen in "Bright's disease"; and second,

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concentric proliferation of fibroblasts in the periglomerular tissues, occasionally associated with proliferative capsulitis or more rarely with slight proliferative glomerulitis. Subsequently hyalinization of the capsule occurs with adhesions to the glomerular tuft. Staemmler and Dopheide [3] and later Pfeiffer [4] describe two types of glomerular lesions in ascending contraction of the kidney; first, a primary thickening of the capsule leading to hyalinization of the glomerulus; and second, focal glomerular adhesions. These are alleged to follow focal desquamation of epithelial cells. Hyalinization of the glomerular loop and finally of the whole glomerulus results and granulation tissue invades the hyaline mass from without. Eventually the affected glomeruli disappear entirely. The authors regard the process as due to a toxin diffusing through the glomerular capsule from without.

Selection of Cases

Fifty-six cases of pyelonephritis were studied from consecutive autopsies performed during 1932, 1933 and 1934 (Tables I and II).* Twenty-nine of these were associated with primary lesions in the genito-urinary tract (prostatic enlargement with urinary retention 11, calculous pyelonephritis 5, prostatic abscess 4, urethral stricture 3, retention of neurogenic origin 3, carcinoma of bladder 1, vesico-vaginal fistula 1, and bilateral hydronephrosis of unknown origin 1). In 26 of these cases bilateral pyelonephritis was present, in 2 the lesion was unilateral.

Of the remaining 27 cases 10 showed gross changes in the ureters, pelves and calyces, *e.g.* dilatation of the pelvis with roughening, thickening and injection of the mucosa. Such signs were absent in 17 cases. These showed evidence, however, of active inflammation and the chronicity of the process was judged by the degree of renal contraction. In the chronic cases of this group a diagnosis of ascending infection could not be made definitely but the diagnosis of pyelonephritis was made on the basis of clinical, macroscopic and microscopic evidence discussed elsewhere.[1] Macroscopically broad flat scars of irregular distribution were taken as characteristic of pyelonephritic contraction; microscopically interstitial infiltration predominating in the medulla was regarded as supporting evidence.

On a histological basis the cases were divided into two main groups

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— acute and chronic. These were again subdivided into focal and diffuse lesions. Since the unilateral involvement of the kidneys could not possibly be causatively related to a general vascular hypertension, we planned these cases under the column of "focal" lesions for the special purpose of this investigation.

Analysis of Data

(1) *Occurrence of Hypertension and Renal Insufficiency in Pyelonephritis*

In acute focal pyelonephritis neither hypertension nor renal failure was encountered. Of 13 patients with acute diffuse pyelonephritis 9 died in uremia and hypertension was present in 4 of these. Two patients presented the picture of essential hypertension with superimposed diffuse acute pyelonephritis but without renal insufficiency.

In chronic pyelonephritis with focal or unilateral contraction of the kidney hypertension was present in 6 out of 9 cases and in 3 of these the individuals died in uremia. From the available evidence it appears that these 6 were cases of "primary" or "essential" hypertension with ischemic contraction complicated by focal pyelonephritis, the latter precipitating the uremic termination in 3 cases.

In chronic pyelonephritis involving the kidneys diffusely, hypertension and uremia were associated in 16 out of 26 cases; hypertension alone was present in 4 and uremia without hypertension in 6. In the majority of instances it is impossible to decide whether we are dealing with a primary "vascular" hypertension or with secondary "renal" hypertension. So far as our present investigation is concerned, however, such a differentiation is not essential.

(2) *Inflammatory Lesions in the Glomeruli in Pyelonephritis*

Ischemic changes in the glomeruli are very common and need no description. Inflammatory lesions, on the other hand, are comparatively rare. It is possible, however, to distinguish two types. The first resembles closely the lesion we have described as "alterative glomerulitis" in decompensated benign nephrosclerosis.[1] The second and more common type appears to be a direct extension of the inflammatory process from the interstitial tissue. Since this form of glomerulitis is given very little attention in the literature we describe

it in some detail. Accumulations of leukocytes and fibrin are frequently found in the periglomerular lymph spaces. In certain glomeruli these can be seen to break through Bowman's capsule into the capsular space (Figs. 1 and 2). The capsule itself may be invaded by leukocytes and its fibrous layer may undergo disintegration. The epithelial cells are lifted off the basement membrane, then degener-

TABLE I

Cases of Pyelonephritis Without Hypertension

Autopsy No.	Age	Sex	N.P.N.	Diagnosis	Tubular dilatation	Alterative glomerulitis
	yrs.					
32-330	58	M	31	Focal acute pyelonephritis	–	–
32-435	27	F		" " "	–	–
32-619	69	M	35	" " "	–	–
32-642	27	M	32	" " "	–	–
33-85	18	F	–	" " "	–	–
33-674	50	F	32	" " "	–	–
34-669	53	F	60	" " "	–	–
34-319	68	M	43	" " "	–	–
32-77	83	M	57	Diffuse acute pyelonephritis	Moderate	–
32-168	57	M	131	" " "	Moderate	–
32-238	72	M	–	" " "	Slight	
32-264	40	M	33	" " "	–	–
32-337	44	F	39	" " "	–	–
33-448	77	M	55	" " "	Slight	
34-417	55	M	70	" " "	–	–
32-494	21	F	60	Focal chronic pyelonephritis	Slight	–
33-583	48	M	27	" " "	Slight	–
34-446	57	M	33	" " "	Slight	–
32-384	74	M	95	Diffuse chronic pyelonephritis	–	
33-378	53	F	167	" " "	Slight	–
34-138	61	M	100	" " "	Moderate	–
34-128	31	F	+	" " "	–	–
34-327	53	F	167	" " "	Slight	–
34-531	22	F	185	" " "	Severe	+

ate and desquamate. Leukocytes migrate into the capsular space and may be seen to encircle a normal looking glomerulus. Acute focal adhesions occur between the capsule and glomerular tuft. The exudate of fibrin and leukocytes can be seen to spread to the adjacent glomerular loop where it may remain localized or may extend to the rest of the glomerulus (Fig. 3). Adjacent to the point of capsular

TABLE II

Cases of Pyelonephritis With Hypertension *

Autopsy No.	Age	Sex	Blood pressure	N.P.N.	Diagnosis	Tubular dilatation	Alterative glomerulitis
	yrs.						
32–481	69	M	100/90	53	Diffuse acute pyelonephritis	–	–
32–482	72	M	180/90	85	" " "	Considerable	–
33–304	72	M	(580 gm.)	54	" " "	Moderate	–
33–698	52	M	170/85	122	" " "	Considerable	+
34–92	41	M	250/160	135	" " "	Moderate	+
34–450	80	M	230/140	71	" " "	Severe	+
32–163	57	M	170/94	38	Focal chronic pyelonephritis	–	–
32–505	63	F	240/120	64	" " "	Considerable	+
32–656	54	F	240/120	120	" " "	Moderate	+
33–284	70	M	(460 gm.)	67	" " "	Considerable	–
33–528	53	M	208/100	48	" " "	Considerable	Only one glomerulus found
33–730	75	M	180/100	45	" " "	–	–
32–68	76	M	230/106	43	Diffuse chronic pyelonephritis	–	–
32–85	68	F	150/100	194	" " "	Severe	+
32–515	55	F	170/70	242	" " "	–	–
32–566	48	F	185/85	140	" " "	Slight	–
32–614	56	M	150/130	41	" " "	Moderate	–
32–626	62	F	200/90	195	" " "	Considerable	–
33–65	67	M	180/65	265	" " "	Considerable	–
33–397	70	M	(460 gm.)	67	" " "	Considerable	+
33–690	78	M	(500 gm.)	122	" " "	Slight	–
34–26	71	F	(570 gm.)	280	" " "	Considerable	–
34–101	39	M	170/110	250	" " "	Severe	+
34–695	66	M	(450 gm.)	89	" " "	Considerable	Only one glomerulus found
34–618	64	M	(500 gm.)	–	" " "	–	–
34–522	54	F	230/136	195	" " "	Considerable	Only one glomerulus found
34–502	32	M	180/110	200	" " "	Considerable	–
34–300	73	M	+	105	" " "	Considerable	–
32–368	55	M	185/85	57	" " "	Slight	+
32–332	70	M	200/94	97	" " "	Considerable	–
34–77	52	M	180/100	120	" " "	Severe	+
34–557	37	F	250/150	200	" " "	Severe	+

* In some cases the blood pressure was not elevated on admission but a previous history of hypertension was present or an increased heart weight was recorded at autopsy (given in blood pressure column).

invasion proliferative glomerulitis, epithelial swelling and increase in leukocytes are occasionally seen confined to one glomerular loop (Fig. 4). In some glomeruli bland adhesions are present which may result from the above process. They can only be distinguished from the adhesions which follow "alterative" glomerulitis if the previous stages of their development can be recognized.

Alterative glomerulitis (Figs. 5 and 6) occurring in pyelonephritis is in no sense to be regarded as a direct extension of the inflammatory process from the interstitial tissue. The necrosis of the capillary loops originates in the glomerular tuft itself and leads to capsular adhesions from within. Capsular proliferation may be seen in both forms of glomerulitis but is a comparatively rare finding.

The distribution of these two types of glomerular lesions is characteristic. The "invasive" form is most common in areas of interstitial infiltration and may be found wherever such infiltration is present whether the process is unilateral or bilateral. The alterative type, on the other hand, may be found where interstitial infiltration is minimal or absent. We have seen this form of glomerulitis in a kidney which showed no other change than gross tubular dilatation in response to complete ascending atrophy of the opposite kidney. It is never present in a unilateral pyelonephritis where the remaining kidney shows no signs of functional impairment.

On comparing the histological and clinical data it appears that the invasive type of glomerulitis is in no way related to the occurrence of either hypertension or renal insufficiency. Alterative glomerulitis, on the other hand, bears a definite relation to these disturbances of function. In our series of 56 cases of pyelonephritis alterative glomerulitis occurred in 13. Twelve of these showed clinical evidence of hypertension and renal insufficiency. In the single exception death occurred in uremia but the blood pressure was normal (140/75 mm. Hg.), and at autopsy the heart weight was 240 gm. The clinical notes record the presence of retinal hemorrhages.

The nitrogen retention in these cases was of true renal origin, *i.e.* was associated clinically with impaired concentrating power of the urine and histologically with tubular dilatation.

Conclusions

Two types of inflammatory lesions of focal distribution occur in the glomeruli in pyelonephritis. The first is peculiar to this condition and results from extension of the interstitial inflammation to the glomerulus. The second or "alterative" type of glomerulitis occurs in pyelonephritic contraction of the kidney as a manifestation of a generalized vascular disease. Clinically it is associated in the overwhelming majority of cases with hypertension and renal insufficiency. Histologically its distribution in the kidney is apparently independent of the interstitial inflammatory process. The lesion itself is indistinguishable from the focal glomerulitis which is found in essential hypertension of the "decompensated benign" or malignant types, in which also it is closely associated with renal insufficiency.

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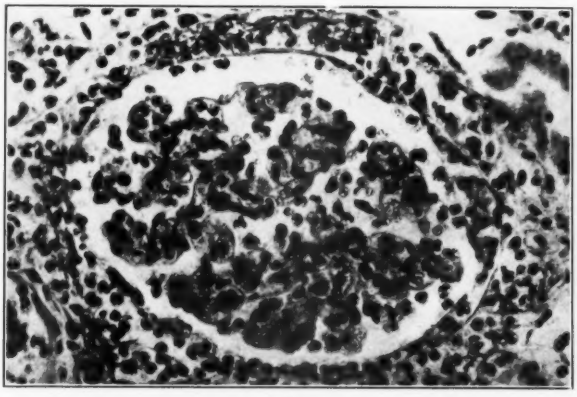
DESCRIPTION OF PLATES

PLATE 13

FIG. 1. "Invasive" type of glomerulitis in pyelonephritis. Accumulation of leukocytes and fibrin in periglomerular lymph space invading Bowman's capsule. Eosin-methylene blue.

FIG. 2. "Invasive" type of glomerulitis. Leukocytes invading Bowman's capsule at one point. Eosin-methylene blue.

FIG. 3. "Invasive" type of glomerulitis; acute adhesions with Bowman's capsule at point where exudate of fibrin and leukocytes invades Bowman's capsule and part of glomerular tuft. Eosin-methylene blue.



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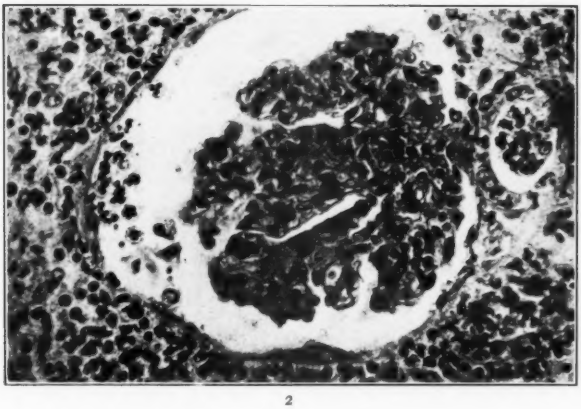

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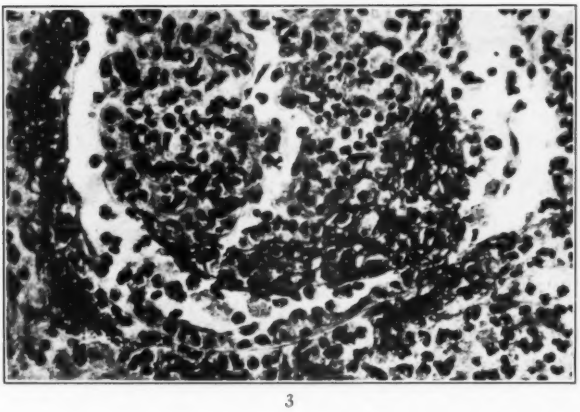

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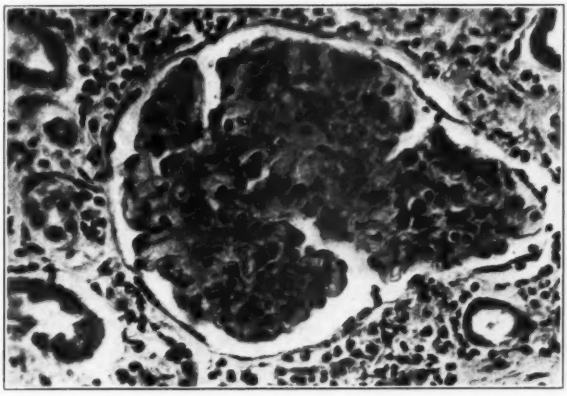
PLATE 14

FIG. 4. "Invasive" type of glomerulitis; epithelial swelling and increase of nuclei in one glomerular loop adjacent to point of invasion. Eosin-methylene blue.

FIG. 5. Alterative type of glomerulitis in pyelonephritis. Adhesive glomerulitis with hyalinization of some loops and still active necrosis in some others. Eosin-methylene blue.

FIG. 6. Alterative glomerulitis in pyelonephritis. Adhesion with early degenerative changes in adjacent glomerular loop. Eosin-methylene blue.



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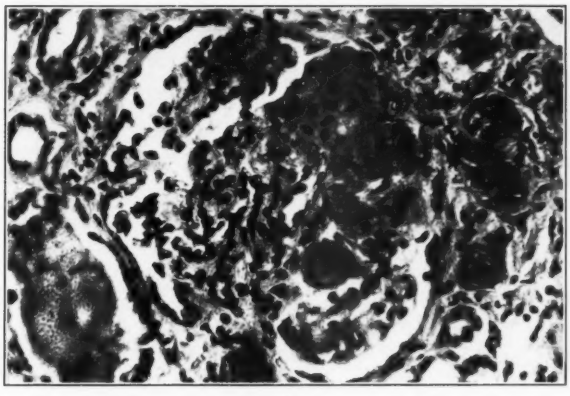

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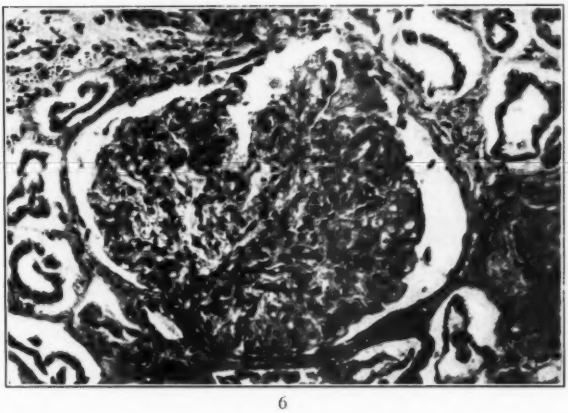

6

A STUDY OF ADRENAL CORTEX MORPHOLOGY *

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In our experimental studies on the physiology of the adrenal gland we looked for morphological criteria whereby we could determine the degree of activity and the state of exhaustion of the adrenal cortex.† The accepted methods did not entirely meet critical analysis. Variations in the amount and distribution of lipoids seemed to depend too much upon the technique of fixation and the care used in embedding and sectioning. The size and weight of the gland also proved to be of limited use in determining its functional state.

MATERIALS AND METHODS

The adrenal glands of normal frogs, mice, rats, guinea pigs, rabbits, cats, dogs, monkeys and humans ‡ have been studied as reference controls. These form the basis for a determination of the changes taking place under abnormal conditions. The latter have been experimentally produced and also observed in naturally occurring infections. A variety of experimental procedures was undertaken in an effort to produce specific cortico-adrenal lesions. Among the substances used were bacterial toxins, thyroxin, parathormone, adrenalin, histamine, mercuric chloride, and extensive body burns were also employed.

Although our own experimental material was prepared for microscopic examination by a variety of special techniques, the majority of adrenal glands which others have given us the opportunity to study were fixed in ordinary fixatives and stained with hematoxylin and eosin. A scheme of identification was gradually developed and tested with many series of "unknown" glands.

* Received for publication July 26, 1935.

† A preliminary paper was read before the American Association of Anatomists, March 1934. *Anat. Record*, 1934, **58**, 43 (No. 4 and Suppl.).

‡ A human adrenal gland that is normal by our standards is very difficult to obtain.

Observations

Before undertaking a detailed description of the morphological changes accompanying certain physiological and pathological states we shall mention briefly our conception of the life history of the adrenal cortex cell. This is discussed in detail in a paper by Zwemer, Wotton, Nussman and Norkus.[1] We agree with Mulon,[2] Bogomolez,[3] Goormaghtigh,[4] and Hoerr [5] that the adrenal cortex grows from without inward, new cells being formed at the periphery and destroyed in the reticular zone near the medulla. That the glomerular cells arise from indifferent connective tissue-like cells in the capsule is our additional contribution. These capsular cells lose their long processes, become short ovals and take up lipoid droplets. A further increase in the amount of cytoplasm and a marked increase in cell fats mark the transition to the spongiocytes. The latter retain and emulsify their fat content as they are gradually pushed inward by the formation of new cells. After an experimentally induced discharge from these cells, lipoid can be demonstrated in the blood stream in the form of small fat droplets by a special gelatin embedding lipoid technique (Zwemer [6]). Direct observation of living frog adrenal glands, under normal and experimental conditions (Singer and Zwemer [7]), strengthens the view that lipoids may be extruded from the cells in droplet form as well as in non-visible secretions.

As the cells secrete, the ratio of cytoplasm to nucleus is greatly diminished, so that the innermost regions of the cell cords consist of rows of nuclei with very small remnants of cytoplasm still surrounding them. In the end stage the cell is represented by a pyknotic nucleus which is finally phagocytozed.

These studies show that the fundamental plan of adrenal cortex cell progression is the same for all species investigated. The differences between normal glands of various species appear to be due to changes in the relative proportions of the cell types. For ease in description we shall limit the detailed discussion to adult mammalian adrenal glands, with particular reference to those of carnivores and primates. The picture, as seen in a segment of a median section of the gland, will be described and illustrated.

A normal gland (Fig. 1) shows the capsule, a single or incomplete double row of glomerular loops, a moderately wide fascicular zone composed of spongiocytes in the outer half and smaller cells in vari-

ous states of discharge in the inner half. Internal to this region and near the medulla is the network of cell cords known as the "reticular zone." In its wide capillary meshes are found many macrophages; these assist in removing the cellular débris of exhausted and incompletely discharged cells.

The first modification of the normal picture reveals an unusually large number of spongiocytes present in the entire fascicular zone. The reticular zone is reduced to a small band of cell cords. This type of gland we have called the lipoid storage type (Fig. 2). A minor modification may show the capsule to be thickened and an increased number of circles and loops of cells is present in the glomerular zone. This latter picture is due to stimulation of the cortico-adrenal cells to hypertrophy and hyperplasia. It may occur as a result of anterior pituitary, mild thyroid, or other treatment.

With an acute demand for cortico-adrenal secretion (Fig. 3) there is a rapid change in the reticular and inner fascicular cords. The outer zones apparently remain unchanged and the adrenal cortex in some cases of death in the acute stage gives the appearance of having an ample store of cortical lipoids. In spite of this apparent store the bodily condition and blood chemistry may indicate a state of adrenal insufficiency and a careful study of sections stained for lipoid shows that the visible fat present is in much larger droplets and often stains differently from normal. This lipoid retention with insufficiency is perhaps a result of the time factor. If we suppose the existence of a polymer of cortin or a precortin, time would be necessary for this substance to be converted into the active hormone. In an acute demand adrenal insufficiency may appear before this change can take place. A very acute demand results in an exhausted gland with little indication of new cell formation (Fig. 4).

When the demand is mild and has existed for only a short period of time, the adrenal morphology is also different (Fig. 5). The glomerular zone is wider, but composed of somewhat smaller cells, spongiocytes have almost disappeared, and the inner fascicular zone is extensive with narrow irregular columns of small cells. The reticular zone does not show the accumulation of débris that is found with acute demand. If the mild demand has occurred for some time the hyperplasia may result in an adrenal that is larger than normal. The cell columns are very narrow and the larger capillaries may be congested (Fig. 6). In spite of an enlarged gland, the animal may suffer

from adrenal insufficiency. One can have a large depleted adrenal in addition to having a large, well stored gland.

A severe but not necessarily acute exhaustion of the adrenal cortex modifies the foregoing picture (Fig. 7). The capsule is thickened and in it accessory or "adenomatous" masses may be embedded. The glomerular zone is wide, quite irregular, and frequently has layers of cells which are crescentic in cross-section, the convexities being directed inward, as found by Hoerr.[5] This directional curve can be seen best in adrenal glands that have been actively stimulated. The fascicular zone is irregular and is composed of small cells having a diminished amount of cytoplasm. The cell columns frequently are widely separated by large capillaries. The reticular débris accumulates faster than it can be removed by macrophages. In this type we see that in spite of the rapid formation of new cells there is little accumulation of lipoid (and its associated hormone). The life history of these small cells is probably extremely short, and an individual with this type of gland can easily be thrown into complete adrenal insufficiency by a slight additional demand for the vital secretion. A small accessory gland that is maintaining life in an experimental animal often has this appearance.

If a period of severe prolonged demand continues until death occurs from uncomplicated adrenal insufficiency, we see an exhausted adrenal (Fig. 8). This type of gland has no definite fascicular zone and there is a rapid transition from the accumulative to the degenerating phases. New cell formation is not sufficiently rapid to keep up with the secretory or discharge phase, and no storage is apparent.*

Discussion

On the basis of our observations we have attempted to describe eight conditions present in adult mammalian adrenal glands. There

* A series of "unknown" adrenals from 200 human autopsies was very kindly put at our disposal by Dr. Seecof and checked by him from his and our records. A comparison of our classification with the pathologists' findings showed a close correlation of adrenal cortex morphology with the functional demands one might expect from the cause of death. In 80 per cent of the cases the group to which the gland belonged was easy to determine; the remaining 20 per cent of the cases were difficult to determine for two reasons. When the adrenals were from very young children the organization of the gland was incomplete. The sections frequently showed rapid multiplication of cells in the capsule and outer glomerular zone and there were also a number of so-called adenomas of cortical tissue. In young adrenals this appearance is due to portions of cortex that have failed as yet to become incorporated within the gland capsule. Another difficult group consisted of adrenals from cases of unusual causes of death.

are, however, gradations between these, and additional complicated types resulting from experimental procedures. An example of complication would be a gland that had been stimulated to hyperplasia and hypertrophy and then subjected to an acute demand.

The changes occurring in the adrenal cortex cells during normal metabolism seem to be accelerated by increased catabolism in other parts of the body.

To determine adequately the condition of a gland under consideration one must, of course, first know the normal proportions of the formative, loaded and discharging types of cells for the species studied. For example, in some species the normal gland seems at first to be composed entirely of loaded spongiocytes and the typical cell sequence becomes more apparent only after experimental procedure. For these species (frog, turtle) the storage type of gland seems to be the normal condition for certain seasons of the year. In other species, particularly among the mammals, there is a more rapid cell utilization, and cells in all stages can be found in a single segment of a median section. Among mammals there is a species difference in the amount of spongiocyte tissue normally found. This has been noted by others and expressed as the amount of visible lipoid found in the outer fascicular zone.

It should be noted that the cell columns in different segments of a single section may vary considerably as a result of intermittent blood circulation. An estimation of the state of the gland as a whole depends, of course, on the condition present in most of the sections. If one wishes to determine minor changes, some commonly used quantitative measurement of relative proportions of cell types may be employed. This may be by cell counts, by polarplanimetric measurement of different cell type areas, or by weighing paper outlines of areas.

One of the fundamental features of the normal adrenal cortex in all types studied is the large cell containing much visible lipoid in a finely divided state. The lipoids are undoubtedly there for some purpose. This point needs no emphasis since the functional state of the gland has long been gauged by its lipoid content. Furthermore, chemical studies have shown that the vital hormone, although somewhat water soluble, is extracted with the lipoids by organic solvents. One can conceive the spongiocyte lipoid to be either the raw substance from which the hormone is made, or the vehicle in which it is

stored. Perhaps both functions are performed by the lipoids. If the hormone were not stored in a protected and relatively inactive form, it might well affect the structure of the cells by which it is formed (*cf.* gastric secretions and postmortem autolysis). By conceding that the spongiocyte is the cortico-adrenal lipoid storage cell, one admits the presence of accumulating and discharging cells. The rapidity with which new cells are formed is one factor in an increased gland size, but the rapid hypertrophy of these new cells as a result of their lipoid storage also contributes to the increase. If lipoids are excessively stored the gland size increases quite rapidly. On the other hand, if the discharging phase predominates, one could expect to find a small gland with very few of the loaded, spongiocyte cells. Rapid cell proliferation, however, might give rise to a large gland. As in the thyroid gland, an increased activity results in a different morphological appearance from that seen in storage, although in both cases the glands may be enlarged.

This broad plan of viewing adrenal cortex changes taking place under natural conditions was applied to specific lesions induced by bacterial and other toxins. In these cases the adrenal gland morphology depended on the extent of the infection, the amount of toxin injected or produced, and the rapidity with which death occurred, *i.e.* the length of time over which the toxin had acted.

No attempt has been made to discuss fully the pathology of the adrenal glands. This has been extensively reviewed by Goldzieher.[8]

Summary and Conclusions

The morphology of the adrenal cortex seems to reflect the demands imposed by body needs.

An excess of hormone over normal requirements is shown by an increase in the number of storage cells.

An acute demand discharges the mature cells but does not immediately affect the accumulative cell types.

Prolonged demands stimulate new cell formation, which may or may not be able to counteract the cell utilization of the discharging phase.

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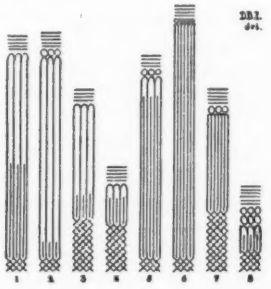
DIAGRAM OF CORTICO-ADRENAL TYPES



The accompanying schematic diagram may aid in an understanding of the plate by indicating the relative proportions of cell types generally present in the different morphological states of the adrenal cortex.

Horizontal lines at the top of a figure indicate the capsule.

Circles and arches are used for the small proliferating new cells (zona glomerulosa).

Vertical lines represent the columns of cells bounded by capillaries (zona fasciculata).

(*a*) If the lines are widely spaced the cells are large and loaded with lipoid droplets (spongiocytes).

(*b*) If the lines are close together the cells are of the active secreting type.

Cross-hatched areas correspond to the inner network of cell cords with its sinusoids, macrophages and cellular débris (zona reticularis).

DESCRIPTION OF PLATE

PLATE 15

FIG. 1. *Normal* secreting adrenal cortex with a balance between accumulating and discharging cell types.

FIG. 2. *Lipoid storage* with the accumulative cells predominating.

FIG. 3. An *acute demand* results in a rapid cytoplasmic diminution in the inner zones.

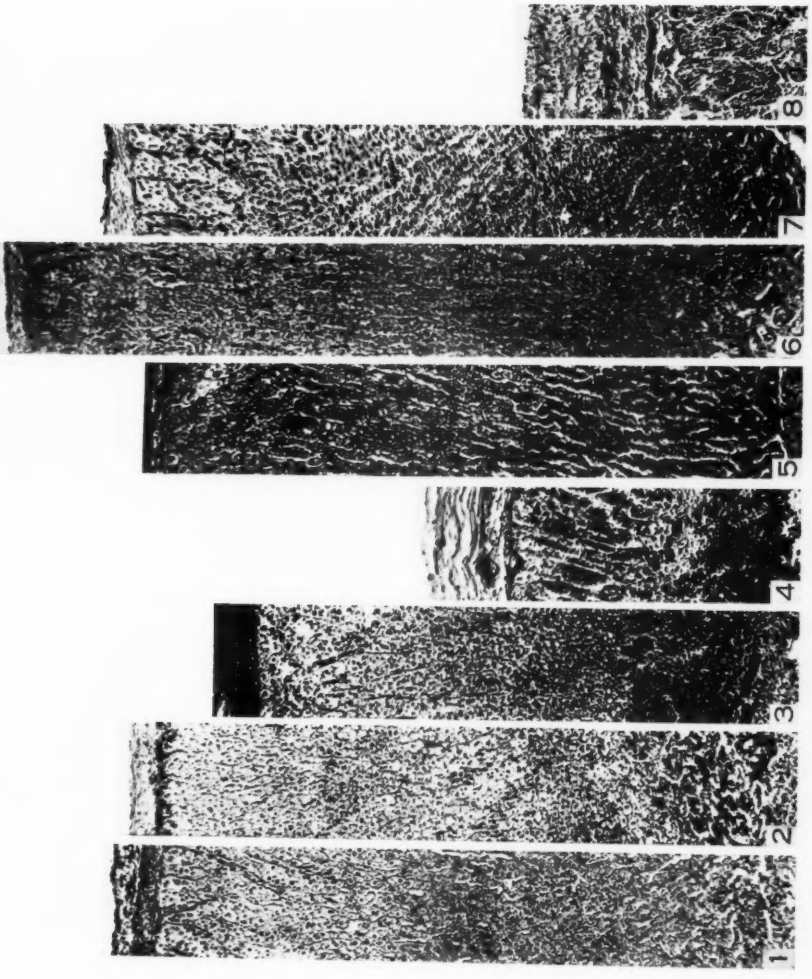
FIG. 4. A *severe acute* demand depletes the gland with little or no indication of new cell formation.

FIG. 5. A *mild stimulation* to discharge is indicated by a decrease in the number of lipoid-loaded spongiocytes.

FIG. 6. *Prolonged activity* is associated with an increase in accumulative and discharging cell types and an absence of lipoid storage cells.

FIG. 7. In a *severe* condition, new cell formation and discharge follow each other rapidly and the normal, regularly arranged cell columns no longer are present.

FIG. 8. *Severe prolongation* of the demand results in an atrophic adrenal with groups of cells separated by fibrous connective tissue which no longer resembles the reticular tissue usually present.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A CHEMICAL ANALYSIS OF ATHEROSCLEROTIC LESIONS IN HUMAN AORTAS *

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The few chemical studies of atherosclerotic aortas which were reported before 1933 are well summarized in Cowdry's [1] survey of arteriosclerosis. In August, 1934, Meeker and Jobling [2] reported analyses for lipids on a series of aortas. In October, 1934, Rosenthal [3] reported analyses for total fat content in aortas and based certain conclusions on the assumption that "the proportions of the fatty constituents remain constant in atherosclerosis." During the spring of 1934 analyses of aortas for lipids were made in this laboratory in connection with a report on atherosclerosis which was being prepared for the annual meeting of the American Heart Association. The results of these analyses are strikingly similar to those of Meeker and Jobling, and do not substantiate Rosenthal's "constant lipid proportion" hypothesis. Also, they do not confirm the earlier work of Schönheimer [4] who found a steadily increasing ratio of ester to free cholesterol with advancing atherosclerosis.

The tissue analyzed in this laboratory consisted of sections of aortas from 11 adults. Since arteriosclerosis is a patchy lesion and may vary in a given aorta from very slight atheroma to far advanced lesions with varying degrees of fibrosis, calcification, ulceration and thrombosis, it seemed far more valuable to analyze individual lesions than entire aortas, as had been done by many earlier investigators. Also, since lesions which differ considerably microscopically often appear very similar to the naked eye, each lesion analyzed was sampled for microscopic study. In order to control to some degree the factors of age and individual variation, several sections were taken from each aorta and included relatively normal appearing areas as well as atherosclerotic lesions.

The sections were obtained at autopsy, care being taken to prevent contamination with any fat-containing substance. The pieces of tissue were washed quickly to remove any clotted blood and blotted

* Received for publication August 2, 1935.

dry with fat-free filter paper. The adventitia was then stripped off in order to remove the adventitial fat. After a little practice it was found possible to make the line of cleavage occur at relatively the same plane in the wall of each aorta. This was checked microscopically. From these adventitia-free portions of aorta several samples were dissected, care being taken that all parts of a given sample should appear uniform. From each sample a representative slice was placed in 10 per cent neutral formalin for microscopic study. From the remainder of each sample several half gram portions were weighed to 1 mg. accuracy and each portion was stored in a small amount of alcohol-ether mixture.

The chemical methods used were essentially those of Bloor [5] and Yasuda,[6] with certain minor modifications because of the tissue used or the equipment available. The lipids were extracted by grinding the sample of aorta in a mortar with a small portion of fat-free ground glass, a few cubic centimeters of distilled water being added to make a paste. Then a small amount of alcohol-ether mixture was added and the grinding continued. From time to time the solute was decanted into a fat-free filter, more solution added to the mortar, and the grinding continued, using successively several small portions of cold alcohol-ether mixture, hot alcohol-ether, ether, and lastly, boiling absolute alcohol. The filtrate at this point was often slightly cloudy, but refiltration produced a clear solution, even though the filter was repeatedly washed with small portions of the same solutions as those used in the first filtration. The filters were saved and the residues from several cases re-extracted together and the filtrates analyzed to make sure that practically all of the lipids were extracted each time.

The final filtrates were collected in 100 cc. volumetric flasks, which were then filled to the mark and aliquots taken for analysis.

Yasuda's modification of Bloor's method for free and total cholesterol was used. It consisted essentially in precipitating the cholesterol with digitonin, freeing the digitonide from other lipids and from excess digitonin, dissolving the isolated digitonide in boiling hot alcohol, evaporating to absence of alcohol, oxidation of the residue with 1 N potassium dichromate, using Nicloux' solution as a catalyst, and finally titrating the excess dichromate with 0.1 N sodium thiosulphate. When determining total cholesterol, the samples were saponified with sodium ethylate before precipitation with

digitonin. The figures for cholesterol esters were obtained by subtracting those for free cholesterol from total cholesterol. Since 10.62 cc. of 0.1 N potassium dichromate are needed to oxidize 1 mg. of cholesterol as digitonide,

$$\frac{\text{cc. of 0.1 N } K_2CrO_7 \text{ used}}{10.62} = \text{mg. cholesterol in aliquot analyzed.}$$

Lecithin was determined by precipitating it with acetone and magnesium chloride, isolating the precipitate and dissolving it in moist ether. The solute was then evaporated and the residue oxidized as for cholesterol. No attempt was made to separate lecithin from the other phospholipids, so the percentages given probably include some cephalin.

The figures for total lipid were obtained by oxidizing together the total fatty acids and total cholesterol in aliquots of the alcohol-ether extract which were saponified, acidified, and extracted with petroleum ether. By subtracting the amount of potassium dichromate used to oxidize the total cholesterol from that used for total lipid the figures for fatty acids were obtained. The latter are not wholly reliable since some of the more volatile fatty acids probably were lost when the petroleum ether extracts were evaporated. There are also other imperfections in the above procedures, but these methods were chosen for this investigation because, in this laboratory, the recovery of lipids from known test samples by these methods has approached more nearly and consistently the theoretical values than when other methods have been used.

The types of cases from which sections of aorta were taken are included in Table I.

In Table II the results of gross, microscopic and chemical studies on the various sections of aortas are listed by cases. The sections from the more and from the less atheromatous areas of the same aorta are identified by the use of a letter following the case number. In all instances two or more samples were taken from each area (A, B, C, D, and so on) of each aorta for analysis. When these duplicate samples differed 0.1 per cent or more the extremes are given.

In each of 6 cases there was a marked difference in the degree of atherosclerosis in the two or more types of samples taken, and in each instance the more atheromatous portions yielded higher percentages of each lipid than the more normal portions of the same

TABLE I

Types of Cases Analyzed

Case No.	Age	Sex	Hours post-mortem	Cause of death	Other findings
1	*yrs.* 54	M	3	Lobar pneumonia	Extensive cellulitis, probable septicemia
2	53	M	6	Lobar pneumonia	Advanced general arterio-sclerosis with cerebral degeneration
3	43	F	4	Confluent lobular pneumonia	Bronchiectasis, chronic biliary disease, general arterio-sclerosis, chronic pyelitis, vestigial right kidney
4	62	F	14	General arteriosclerosis with cerebral softening	Coronary sclerosis with myo-cardial degeneration
5	54	M	5	Urethral stricture with pyelonephritis	Generalized arteriosclerosis
6	29	F	6	Confluent lobular pneumonia	Tubo-ovarian abscess with pelvic peritonitis, arrested pulmonary tuberculosis, left leg amputated in childhood
7	47	F	6	Intestinal obstruction	Toxic adenoma of thyroid, healed hysterectomy wound
8	50	F	9	Lobular pneumonia	Cirrhosis of liver, exopthalmic goiter with thyroid heart disease, healed panhyster-ectomy wound
9	75	M	4	Lobular pneumonia with empyema	Bronchiectasis, adenoma of the prostate
10	74	M	5	Lobular pneumonia	Epidermoid carcinoma of bladder, chronic biliary disease
11	50	M	6?	Gunshot wound with severance of common carotid artery and fatal hemorrhage	Essentially normal

TABLE II

Morphological and Chemical Findings

Case No.	Area	Gross plaques	Ulceration	Calcification	Lipoid cells	Pooling of lipids	Increased connective tissue	Thinning of media	Inflammatory reaction	Lipoid crystals	Total cholesterol	Free cholesterol	Cholesterol esters	Fatty acids	Lecithin	Total lipid*
											per cent	*per cent*	*per cent*	*per cent*	*per cent*	
1	C	o	o	o	+	o	+	o	+	o	1.0	0.7	0.3	2.3– 3.6		0.12–0.17
	E	+	o	o	+	+++	+++	++	+	+	2.0–2.6	0.7–1.2	1.3–1.4	12.7–14.2		0.53–0.61
2	B	o	o	o	+	o	+	o	o	o	1.7–1.8	1.5	0.2	4.4	0.5	0.13–0.22
	A	++	o	+	+	+++	+++	++	+	+	2.6–3.3	1.7	0.9–1.6		0.9	
3	B	o	o	o	+	o	++	+	o	o	1.0–1.4	0.3–0.4	0.6–1.2	2.7–4.0	0.7	0.14–0.20
	C	++	o	o	+	+++	++	++	++	+	2.2–2.9	1.0–1.2	1.1–1.7	2.1–3.2	0.7	0.19–0.20
	A	+++	+	++	+	++++	+++	+++	++	++++	3.6–4.1	2.1–2.2	1.5–2.0	5.6–7.4	1.8	0.34–0.43
4	B	o	o	o	o	o	o	o	o	o	0.4–0.6	0.3	0.1–0.3	0.7–0.9		0.04–0.06
	A	+	o	+	+	+	++	+	o	o	2.0–2.3	0.9–1.1	1.0–1.3	5.0–5.1		0.26–0.27
5	B	o	o	o	+	o	+	o	o	o	0.5–0.6	0.3–0.4	0.2	0.2–0.6	0.2–0.6	0.03–0.05
	A	++	o	+	+	+++	++++	+++	+	++	2.5–3.4	1.6	0.9–1.8	2.1–3.1	0.9–1.2	0.21
6	B	o	o	o	+	o	+	o	o	o	0.4–0.5	0.3	0.1–0.2	1.6–1.8	0.2–0.3	0.07–0.09
	A	+++	o	++	+	++++	++++	+++	+++	++	2.4	1.8–1.9	0.5–0.6	6.7–7.9	0.5–0.7	0.34
7	A	+	o	o	+	+	++	+	o	o	1.8–1.9	0.4–0.7	1.1–1.5	7.0		0.33
8	A	o	o	o	+	o	+	o	o	o	1.4	1.0–1.1	0.3–0.4	5.1	0.4	0.24
	B	o	o	o	+	o	+	o	o	o	1.8	1.5	0.3	2.9–6.4	0.6–1.0	0.17–0.30
9	B	o	o	o	+	+	+	o	+	o	1.6–1.7	0.4	1.2–1.3	0.4–0.8	1.4–1.8	0.08–0.09
	A	++	o	+	+	+++	+++	++	+	++	2.1–2.3	0.7–0.8	1.4–1.5	5.4–6.5	1.4–1.8	0.28–0.33
10	A	+	+	o	+	++++	++	++	++++	++++	5.5	3.6–4.1	1.4–1.9	16.1–16.7		0.64–0.89
11	A	o	o	o	+	o	+	o	o	o	1.2–1.5	0.3	0.9–1.2	3.1–3.4		0.17
	B	+	o	o	+	+	+	o	o	o	1.6–1.9	0.6	1.0–1.3	4.1–4.5		0.22

* cc. 0.1 N K_2CrO_7 required to oxidize lipids from 1 mg. aorta.

aorta. In 2 other cases (Nos. 3 and 9) this was also true, with the exception of lecithin and fatty acids in the one case, and lecithin in the other. In 2 cases (Nos. 7 and 10) analyses were completed on only the more atheromatous samples. In case No. 8 the types of samples taken appeared to be similar morphologically but were from widely separated areas of aorta; no significant difference in the amount of lipids was found in these samples.

Therefore, it may be concluded that portions of a given aorta which appear alike microscopically are also similar chemically in so far as their lipid content is concerned, and that areas presenting more lipids microscopically contain more lipids chemically. These facts indicate that the atheromatous process in the aorta is essentially different from so-called fatty "degeneration" in such organs as the heart and kidneys where lipids may seem to be increased, as determined by microscopic methods, but are the same in amount, or even decreased, chemically.

In Table III the samples of aortas are arranged according to the degree of atherosclerosis morphologically, without regard to cases. Considering both morphological and chemical data, the samples of aortas seem to fall into five main groups:

Group I. A section of aorta from a female 62 years of age, which showed no evidence of atherosclerosis either grossly or microscopically (Fig. 1).

Group II. Eight sections of aortas in which the lesions consisted of infiltration of the intima with lipoid cells, and not more than a moderate increase in connective tissue. In these sections there were no gross plaques, no ulceration or calcification, no breaking of lipoid cells with pooling of lipids, and no discernible lipoid crystals. In only one section was there microscopic evidence of thinning of the media and this was accompanied by a moderate increase in intimal connective tissue (Fig. 2).

Group III. Four sections in which there was beginning pooling of lipids in the intima. In three of these the gross lesion was a plaque. In the one showing no plaque the fatty acid content was unusually low, considering the type of lesion. In these sections thinning of the media was again found in those areas which presented the greatest increase of intimal connective tissue (Fig. 3).

Group IV. Six sections of gross plaques, four of them calcified but none ulcerated, which microscopically showed marked pooling

of lipids and increased intimal connective tissue. In all of them lipoid crystals were found and in all there was some inflammatory reaction (Fig. 4).

Group V. Two sections of plaques similar to those in Group IV, but with the addition of ulceration and much more numerous lipoid crystals (Fig. 5).

All of the figures for lipids in Table III, except those for total lipid, indicate per cent of wet weight of aorta. Since the proportion of each lipoid substance represented in the "total lipid" is not known, the oxidation value for the latter cannot be reduced to percentage. Therefore, the figures in Table III for total lipid indicate cubic centimeters of 0.1 N potassium dichromate used to oxidize all of the lipids extracted from 1 mg. of aorta.

In order to be able to compare more readily the percentages of lipids in the various types of lesions, the mean and its standard deviation for each lipid in each of the five groups were calculated. As values for the frequencies, all determinations on all aortas in the group were used, in calculating the mean for that group. The mean for each group was also calculated by using the average percentage for each aorta as the value of each frequency. The results of the two methods of calculation were so similar that only one set of figures is given (Table IV).

Meeker and Jobling,[2] when reporting their analyses, did not list their results for each lipid as per cent of wet weight of aorta, but such percentages are readily obtainable from their figures by multiplying lipid per cent of fatty extract by extract per cent of wet tissue. This was done and the mean for each lipid was calculated. Table IV shows the close similarity of results obtained in the two laboratories.

The differences in terminology are readily explained by the fact that the diagnoses of Meeker and Jobling were based on gross examination, while our terms refer to microscopic morphology. Thus, the lesions which we designate as "slight atherosclerosis" appeared normal in the gross, except for slight yellowish discoloration. The "early lesions" of Meeker and Jobling were described by them as being "small, raised, yellowish, opaque, glistening plaques," which description corresponds to the gross appearance of our "moderate atherosclerosis."

Although the variations in the percentages within the groups, as obtained in both laboratories, are too large to attach exact mathe-

Table III

Degree of Atherosclerosis

Case No.	Area	Gross plaques	Ulceration	Calcification	Lipoid cells	Pooling of lipids	Increased connective tissue	Thinning of media	Inflammatory reaction	Lipoid crystals	Total cholesterol	Free cholesterol	Cholesterol esters	Fatty acids	Lecithin	Total lipid *	Age	Sex	Group
											per cent	*per cent*	*per cent*	*per cent*	*per cent*		*yrs.*		
4	B	o	o	o	o	o	o	o	o	o	0.4–0.6	0.3	0.1–0.3	0.7–0.9		0.04–0.06	62	F	I
6	B	o	o	o	+	o	+	o	o	o	0.4–0.5	0.3	0.1–0.2	1.6–1.8	0.2–0.3	0.07–0.09	29	F	
5	B	o	o	o	+	o	+	o	o	o	0.5–0.6	0.3–0.4	0.2	0.2–0.6	0.2–0.6	0.03–0.05	54	M	
1	C	o	o	o	+	o	+	o	+	o	1.0	0.7	0.3	2.3–3.6		0.12–0.17	54	M	
11	A	o	o	o	+	o	+	o	o	o	1.2–1.5	0.3	0.9–1.2	3.1–3.4		0.17	50	M	II
8	A	o	o	o	+	o	+	o	o	o	1.4	1.0–1.1	0.3–0.4	5.1	0.4	0.24	50	F	
3	B	o	o	o	+	o	++	+	o	o	1.0–1.4	0.3–0.4	0.6–1.2	2.7–4.0	0.7	0.14–0.20	43	F	
2	B	o	o	o	+	o	+	o	o	o	1.7–1.8	1.5	0.2	4.4	0.5	0.13–0.22	53	M	
8	B	o	o	o	+	o	+	o	o	o	1.8	1.5	0.3	2.9–6.4	0.6–1.0	0.17–0.30	50	F	
9	B	o	o	o	+	+	+	o	+	o	1.6–1.7	0.4	1.2–1.3	0.4–0.8	1.4–1.8	0.08–0.09	75	M	
11	B	+	o	o	+	+	+	o	o	o	1.6–1.9	0.6	1.0–1.3	4.1–4.5		0.22	50	M	III
7	A	+	o	o	+	+	++	+	o	o	1.8–1.9	0.4–0.7	1.1–1.5	7.0		0.33	47	F	
4	A	+	o	+	+	+	++	+	o	o	2.0–2.3	0.9–1.1	1.0–1.3	5.0–5.1		0.26–0.27	62	F	
9	A	++	o	+	+	+++	+++	++	+	++	2.1–2.3	0.7–0.8	1.4–1.5	5.4–6.5	1.4–1.8	0.28–0.33	75	M	
1	E	+	o	o	+	+++	+++	++	+	+	2.0–2.6	0.7–1.2	1.3–1.4	12.7–14.2		0.53–0.61	54	M	
3	C	++	o	o	+	+++	++	++	++	+	2.2–2.9	1.0–1.2	1.1–1.7	2.1–3.2	0.7	0.19–0.20	43	F	IV
5	A	++	o	+	+	+++	++++	+++	+	++	2.5–3.4	1.6	0.9–1.8	2.1–3.1	0.9–1.2	0.21	54	M	
2	A	++	o	+	+	+++	+++	++	+	+	2.6–3.3	1.7	0.9–1.6		0.9		53	M	
6	A	+++	o	++	+	++++	++++	+++	+++	++	2.4	1.8–1.9	0.5–0.6	6.7–7.9	0.5–0.7	0.34	29	F	
3	A	+++	+	++	+	++++	+++	+++	++	++++	3.6–4.1	2.1–2.2	1.5–2.0	5.6–7.4	1.8	0.34–0.43	43	F	V
10	A	+	+	o	+	++++	++	++	++++	++++	5.5	3.6–4.1	1.4–1.9	16.1–16.7		0.64–0.89	74	M	

* Expressed as cc. 0.1 NK_2CrO_7 required to oxidize lipids from 1 mg. aorta.

TABLE IV

Mean Percentages of Wet Weight of Aortas

Tissue	Total cholesterol		Free cholesterol		Cholesterol esters		Fatty acids	Phospholipids		Ratio of free to ester cholesterol	
	Z.†	M. & J.†	Z.	M. & J.	Z.	M. & J.	Z.	Z.	M. & J.	Z.	M. & J.
No atherosclerosis	0.5	0.4	0.3	0.2	0.2	0.2	1.0		0.3	1.5	1.0
Slight atherosclerosis	1.2		0.7		0.5		3.0	0.6		1.4	
Moderate atherosclerosis (grossly, early lesions *)	1.9	2.1	0.7	0.7	1.3	1.4	4.9	1.6	0.9	0.54	0.56
Marked atherosclerosis (grossly, medium lesions *)	2.6	3.4	1.3	1.3	1.3	2.0	6.5	1.1	1.2	1.0	0.73
Far advanced atherosclerosis with ulceration (grossly, late lesions *)	4.8	4.3	3.0	2.1	1.7	2.1	11.5	1.8	1.4	1.8	1.04

* The gross diagnoses are those used by Meeker and Jobling.

† The initials refer to the authors Zeek,[7] and Meeker and Jobling [2] respectively.

matical significance to the calculated means, yet certain definite trends are shown by these figures, which add a little to our very meager knowledge of the process arteriosclerosis. (1) The percentages of total cholesterol and fatty acids vary directly with the severity of the lesions. (2) The chemical increase in total cholesterol and fatty acids begins with the earliest departures from the normal morphology. The accumulation of lipids in the intima does not represent a secondary regressive change in a fibrotic intimal plaque which has become too large for its blood supply, as has often been asserted, because the chemical increase in lipids begins before there is any plaque morphologically. (3) There is no assurance that all of the additional lipids found chemically in these lesions are visible microscopically as lipids. It is a well known fact that lipids may be increased or decreased chemically in lesions without a corresponding change morphologically. However, if it is assumed that the additional chemical lipids are contained in the added morphological elements it will be noted that the cholesterol of the infiltrating lipoid cells and loose connective tissue must occur chiefly as cholesterol esters, since the increase in cholesterol esters during the early stages of the lesions is much more marked than the increase in free cholesterol. However, when large pools of lipids form in the intima, and especially when these pools ulcerate into the lumen and are no longer sealed off from the lumen blood stream, the increase in free cholesterol becomes predominant and the ratio of free to ester cholesterol rises until it equals or exceeds that of adult normal aortic tissue. This increase in the proportion of free cholesterol in late lesions has been stressed by Meeker and Jobling, but is contrary to the findings of Schönheimer.

This investigation has not revealed the source of the lipids which appear in atheromatous aortas; however, the changing cholesterol ratios point definitely to some process other than simple imbibition from the lumen blood stream. Against this too long unquestioned hypothesis there also is accumulating evidence which arouses doubts as to the rôle which high blood lipids can play in causing atherosclerosis. Examples of such evidence come from autopsies on cases of lipoid nephrosis in which there is often extreme hypercholesterolemia without marked atherosclerosis. The deposition of cholesterol in the wall of the aorta, as well as in the viscera, of herbivorous animals after cholesterol feeding in quantities which they cannot

metabolize, does not produce lesions entirely comparable to those of human atherosclerosis. Perhaps the hypercholesterolemia found occasionally in the late stages of human atherosclerosis may be due to the rupture of atheromatous ulcers with the subsequent discharge of lipids into the blood, and the high lipid content of the blood thus be an effect rather than a cause of atherosclerosis.

An outstanding need at present is a reliable quantitative method for elastin determinations. The disintegration of elastic tissue in atherosclerotic lesions has been observed for a long time morphologically. Does elastin decrease chemically as lipids increase? Pure elastin probably never has been isolated. It is supposedly the protein residue left after all the albumins, globulins and collagen have been removed. In 1911 Selig [8] attempted to compare the elastic content of normal and sclerotic aortas. His methods were crude and he probably did not separate completely elastin from collagen, but he found that with increasing atheroma in the aorta the "protein residue" decreased from over 40 per cent in normal aortas to 10.76 per cent in the most atheromatous ones. In 1913 Ameseder [9] attempted to study the elastin of normal and atheromatous aortas by analyzing for the primary elements composing it, namely, C, H, N, O, and S. He found no significant differences between the normal and the atheromatous vessels in the proportions of these elements. These analyses should be repeated with the more reliable methods now available. If there is no change in the amount of the elements composing elastin as the latter decreases, is it possible that the degenerating, disappearing elastin is converted into lipids? It has been claimed by Walker,[10] Beebe and Buxton,[11] Wells,[12] and others that bacteria can convert proteins into fats, but so far, all attempts to prove that such a conversion takes place in the human body have been unconvincing.

Summary and Conclusions

1. Samples from normal and atheromatous areas of aortas from 11 adult human autopsies were analyzed quantitatively for lipids, and the results compared with microscopic sections from the same areas.

2. The morphological increase in lipids during the progress of atherosclerosis in the aorta was found to be accompanied by a corre-

sponding progressive chemical increase in lipids, including total cholesterol, free cholesterol, cholesterol esters, fatty acids, lecithin and total lipid.

3. The ratio of free to ester cholesterol was found to decrease during the early stages of the process but showed a marked increase in the advanced lesions. This confirms the conclusions of Meeker and Jobling,[2] but is contrary to that of Schönheimer.[4]

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DESCRIPTION OF PLATE

PLATE 16

Types of intimal lesions in aortas analyzed. Verhoeff's elastic tissue stain.

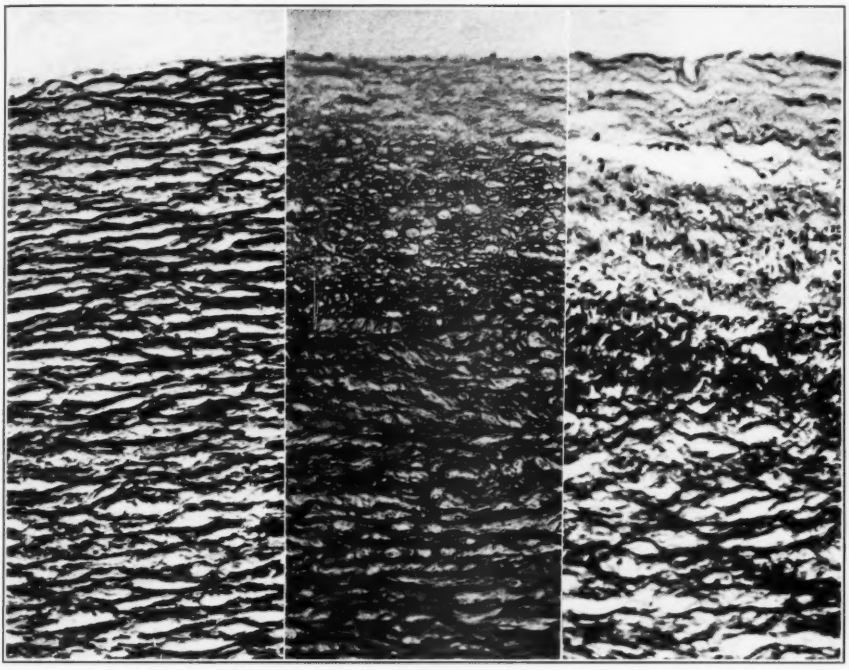
FIG. 1. Case 4, Area B. No atherosclerosis. × 175.

FIG. 2. Case 5, Area B. Slight atherosclerosis. × 175.

FIG. 3. Case 7, Area A. Moderate atherosclerosis. × 175.

FIG. 4. Case 3, Area C. Marked atherosclerosis. × 75.

FIG. 5. Case 3, Area A. Far advanced atherosclerosis with ulceration. × 100.



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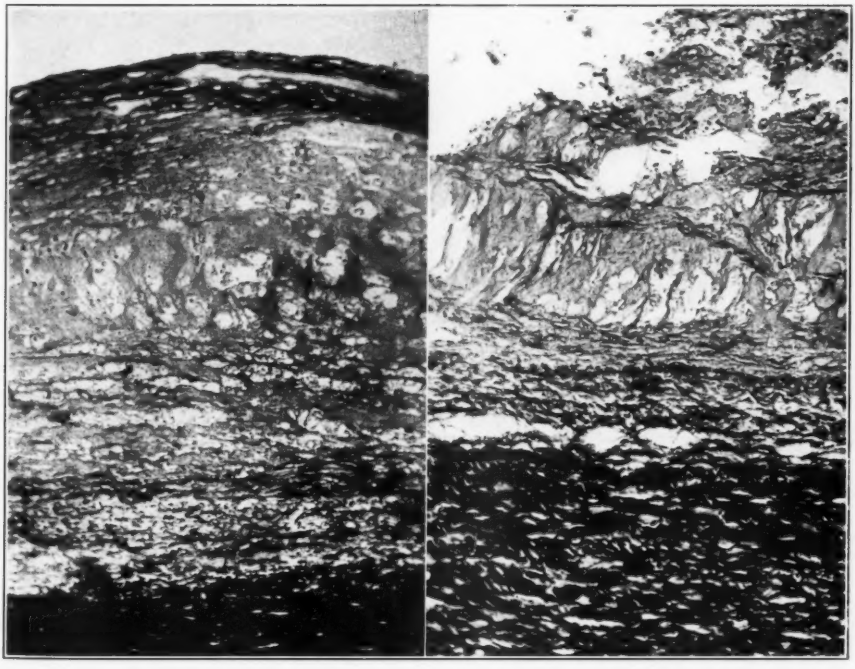

4 5

TREPONEMA PALLIDUM IN SYPHILITIC AORTIC VALVULITIS OF A CONGENITALLY BICUSPID VALVE WITH SUBAORTIC STENOSIS *

Report of a Case

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Syphilis of the aortic commissures, associated with mesaortitis, is the usual type of syphilitic heart disease, and lesions do not occur in the body of the valve except by direct invasion from contiguous structures. Only eight adequately described instances of gummatous endocarditis of the aortic valve resulting from invasion of the cusps by a syphilitic process in the root of the aorta, or from a gumma of the interventricular septum, were found in the literature. Involvement, by similar processes, of the pulmonary, mitral and tricuspid valves has been described five, four and three times, respectively, with a histological description of the diseased valve in 10 of these 20 cases (see Table I). A record of the demonstration of the *Treponema pallidum* in a heart valve was not found.

The basis of this communication is a description of a case of gummatous endocarditis of the aortic valve, in which *Treponemata pallida* were demonstrated, and a review of the cases of syphilitic endocarditis found in the literature. In addition to the syphilitic lesions, a congenitally bicuspid aortic valve and subaortic stenosis were present. The concomitance of these two anomalies has been reported in but 3 cases.[1]

Report of Case

Clinical History: The patient was a 45 year old, white male with clinical evidence of severe aortic stenosis and slight insufficiency, believed to be due to rheumatic disease. Over the upper part of the precordium, maximal in the aortic area, there was a prominent systolic thrill; in the same areas there was a very loud systolic and a blowing early diastolic murmur. The blood pressure was 105/70. A congenital cardiac anomaly was not suspected. There was moderate cardiac decompensation and bronchopneumonia. Substernal pain on exertion and dyspnea appeared 6 weeks before death. The history suggested the pres-

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ence of a valvular cardiac lesion even at the time he contracted syphilis, 15 years before death. The only known antisyphilitic therapy had been six injections of arsphenamine 8 years after the initial lesion. At that time the cardiac signs were similar to those observed during the terminal illness. A history of rheumatic fever was not obtained. The blood and spinal fluid Wassermann reactions were positive. Blood culture was not made and an electrocardiogram was not taken. The temperature was normal.

Postmortem Examination

The autopsy, limited to the thorax and abdomen, was performed 31 hours after death.

The heart weighed 525 gm. The epicardium and subepicardial fat were normal. The coronary arteries, which were not tortuous, contained a few small plaques of thickened intima. There were no adhesions in the coronary sulcus. The myocardium showed no areas of scarring. The left ventricle was hypertrophic. All four chambers appeared to be moderately enlarged and contained no ante mortem thrombi. The tricuspid orifice measured 12 cm. and the pulmonic 6 cm. in circumference. The leaflets of both were normal.

The mitral orifice measured 8.5 cm. in circumference. The anterior leaflet was slightly thickened at its free margin, but was not retracted or vascularized. There were no verrucae. The posterior leaflet was normal. The chordae tendineae were slightly thickened and shortened; a few were adherent to each other at their papillary and valvular attachments. The apices of the papillary muscles were slightly atrophic and the endocardial surface was thickened. Scars were not found on section. There was no wrinkling or roughening of the endocardial surface of the posterior wall of the left atrium.

Located below the aortic valve was an elliptical opening, 1.8 by 1 cm., formed by a ring of gray, fibrous tissue, approaching the consistence of cartilage. This ledge, 5 to 6 mm. wide and 2 to 3 mm. thick, was located from 8 to 12 mm. below the base of the aortic cusps at the level of the junction of the ventricular muscle with the fibrous portion of the heart. The base was circumferentially continuous on either side with the mural endocardium of the left ventricle and the ventricular surface of the anterior mitral leaflet. The endocardium between the aortic valve and the subaortic ring was gray, thickened and slightly wrinkled, but contained no endocardial pockets. At the level of this ring there was an abrupt change to a slight

degree of endocardial sclerosis, in the outflow tract. The undefended space was thin, translucent and bulged slightly into the right ventricle.

The aortic valve admitted the tip of the index finger. The orifice measured 8.3 cm. in circumference. An enlarged posterior cusp, located approximately 1 cm. below the level of the margin of the other two cusps, was 2 cm. long and 0.4 cm. thick, and occupied half of the valve orifice as an almost perpendicular, firm shelf of lusterless, reddish gray tissue. The distal four-fifths of the cusp were vascularized and less firm than the proximal portion. There was fusion of the right and left aortic cusps at the anterior commissure; they were of equal size, moderately thickened and retracted, and the edges were rolled outward. Rising above each commissure in the aorta was a bluish white, semicircular, hyaline plaque about 9 mm. in diameter, the surface of which was raised and slightly corrugated. At the posterior commissures these plaques produced moderate separation; the commissural extremities of the posterior cusp were prominent. The hyaline plaque above the anterior commissure did not produce separation but was continuous with a small firm nodule, to which was attached the conjoint segment formed by the fusion of the right and left cusps, along 8 mm. of their margins and 5 mm. of their valve surface. This segment contained four small linear fenestrations. The nodule and margin of the fused cusps formed the raphé of a bicuspid valve. The attachment of the raphé to the sinus wall was slightly above that of the other two commissures. The fusion, firmness and retraction of these two cusps, in addition to the abnormal position and rigidity of the large posterior cusp, produced a slight degree of stenosis in a markedly incompetent valve.

The sinuses of Valsalva were of usual size and contained a few gray and yellow intimal plaques. The ostium of the right coronary artery was narrowed by a semicircular intimal plaque, but the left orifice was normal. The aorta was not dilated. The first portion contained a few areas of puckering and longitudinal wrinkling of the intima extending into the media. Throughout the aorta there was moderate arteriosclerosis.

The remaining significant pathological changes were: severe passive hyperemia of the lungs and abdominal viscera, bilateral hydrothorax and bronchopneumonia.

Microscopic Examination

Blocks of the heart were cut according to the standard method of Gross, Antopol and Sacks.[2] Additional blocks were cut wherever indicated. Sections were stained with hematoxylin and eosin and in duplicate with Weigert's or Verhoeff's elastic and Van Gieson's connective tissue stains. The author is indebted to Dr. C. V. Weller, of Michigan University, for the Warthin-Starry stains, which were done by a slight modification of the method published in 1930 by Farrier and Warthin.*

The terminology of the valve structure is that employed by Gross and Kugel.[3]

The histological study of the other organs only confirmed the gross diagnoses and revealed no evidence of syphilis.

Aorta: The intima of the ascending portion showed moderate, irregular thickening by fibrous tissue, with the formation of a few small atheromata. The media was the seat of perivascular fibrosis and cellular infiltrations; the elastica was disorganized and interrupted. The syphilitic process had caused narrowing of the mouth of the right coronary artery. The descending aorta presented only the changes of arteriosclerosis. Warthin-Starry stains showed an occasional treponema in the supravalvular portion.

Posterior Commissures: The same severe type of lesion described in the ascending aorta was present, and in addition a few areas of necrosis and fibrocalcific changes. Numerous treponemata were found in the right commissure, valve ring and annulus near the commissural attachment of the posterior cusp.

Bicuspid Commissure; Right and Left Aortic Cusps: There was a raphé composed of dense, avascular fibrous tissue, and areas of elastic fibers cut in various planes and angles, giving a distinct whorled appearance. In addition there were a few areas of laminated elastic and fibrous tissue resembling aortic media. The subendothelial elastica on the surface of the commissure was continuous with the

* Farrier, R., and Warthin, A. S. A study of the effect of Ph upon the third improved Warthin-Starry method for demonstrating Spirocheta Pallida in single sections. *Am. J. Syph.*, 1930, **14**, 394–401.

In the method given on page 400 the following changes were used: procedures 5 and 6, 1 per cent solution of silver nitrate (previously 0.5 to 1 per cent solution); procedures 7 and 8, 55° C. (previously 45° C.); procedures 8 and 10, 3 per cent hydroquinone solution (previously 5 per cent); procedure 13 was omitted.

elastica of the aortic intima. These features revealed the congenital origin of this malformed segment. Syphilitic changes were also found here.

The right and left aortic cusps which were irregularly shortened and thickened by fibro-elastic tissue showed no inflammatory changes.

Posterior Aortic Cusp: In its proximal half was a large area of coagulation necrosis almost surrounded by fibro-elastic tissue containing epithelioid cells, plasma cells, small and large mononuclear cells and an occasional multinucleated cell. Many of the mononuclear cells contained blood pigment (Fig. 1). Adjacent to this gumma was a smaller area of coagulation necrosis. The proximal fourth and base of the cusp showed a syphilitic process obviously of a more chronic nature, characterized by fibrosis and distortion by large vascularized scars, perivascular cellular accumulations and obliterating endarteritis. There were a few, small scattered areas of calcification. The central portion of the distal half was composed of very edematous, vascularized, hemorrhagic and acellular, fibrous and elastic tissue, and a few inflammatory cells. The subendothelial tissue was less edematous and contained many capillaries, fibroblasts, multinucleated cells, plasma and lymphoid cells. Typical treponemata were demonstrated in large numbers in the distal two-thirds and occasionally in the remainder of the cusp and valve ring (Fig. 2). A Gram stain showed no organisms.

Subaortic Ridge: Sections at various places showed an endothelial covered ridge of dense, avascular, almost acellular, hyalinized fibrous tissue containing no inflammatory cells or blood pigment. The intact ventricular elastica beneath the base of this stenotic ledge clearly indicated its congenital origin.

Section Including Posterior Aortic and Anterior Mitral Leaflets and Subaortic Ridge: Inflammatory changes extended from the aorta into the base of the posterior aortic cusp and into the valve ring, the annulus fibrosis and tissue immediately behind it, down toward the anterior mitral leaflet, but did not reach the ring of this valve. In this section treponemata were found only in the aortic valve ring. The mitral leaflet presented minor changes consisting of slight thickening with a bulbous tip composed of whorls of dense fibro-elastic tissue in which there was a single small blood vessel. Inflammatory cells were absent.

Myocardium: There was severe interstitial fibrosis in the subepicardial tissue adjacent to the larger coronary arteries. Perivascular fibrosis was present throughout the myocardium but the vessels were uninvolved by intimal proliferation or other changes. Aschoff bodies were absent. Warthin-Starry stains of the sections showing the greatest degree of fibrosis were negative.

Other Sections: The posterior mitral, the tricuspid and pulmonic leaflets and valve rings were normal. Inflammatory changes were absent in the pulmonary artery. The papillary muscles showed moderate interfascicular and perivascular fibrosis.

Pathological Summary

Acquired Lesions: Syphilitic mesaortitis with narrowing of the right coronary ostium; extension of the process into the right and left posterior commissures and the bicuspid commissures of the aortic valve, and from the aortic root into the posterior aortic cusp, producing severe acute and chronic gummatous endocarditis; interstitial and perivascular fibrosis and hypertrophy of the myocardium. Treponemata were demonstrated in the posterior aortic cusp, in its valve ring, and in the ascending aorta.

Congenital Anomalies: Congenitally bicuspid aortic valve and subaortic stenosis.

Discussion

In syphilis of the aortic valve the inflammatory changes are nearly always restricted to the commissural extremities of the cusps,[4,5,6,7,8] while the free border and body of the leaflets show fibrous, avascular, relatively acellular thickening. The absence of inflammatory changes in the midportion of the cusp serves as a diagnostic distinction from rheumatic valvulitis. When both the commissures and midportion of an aortic cusp are involved by inflammatory changes, a combination of syphilitic and rheumatic disease should be considered.[8] In a rare instance, however, the midportion of an aortic cusp, usually the posterior one, may be involved by extension of an intense syphilitic process of the root of the aorta into the valve ring and base of the cusp, also possibly by a horizontal diffusion from the commissures.[6,7] These are the "ascending" and "descending" types of syphilitic valves described by Benedict.[9] Rarely the syphilitic process in the

root of the aorta may, with or without involving the base of an aortic cusp, extend downward into the anterior mitral leaflet.[10, 11-m, 11-l]

In a gummatous lesion of a cardiac valve the diagnosis of syphilis is evident, even with a negative spirochete stain. Since rheumatic valvular disease is more common than syphilitic, it is necessary, in the absence of giant cells, coagulation necrosis and a positive spirochete stain, to trace the inflammatory changes in the body of a valve leaflet to a syphilitic process in a contiguous structure, usually the aorta or pulmonary artery, in order to demonstrate a presumptive syphilitic etiology.

In the present case the relatively chronic syphilitic process in the proximal third of the posterior aortic cusp was continuous with similar changes in the adjacent commissures, valve ring and root of the aorta, but did not reach the ring of the mitral valve. The most unusual feature is the superimposed acute exacerbation of the syphilitic process in the distal two-thirds of the cusp, and the demonstration of large numbers of treponemata. The latter lesion represents an active syphilitic endocarditis, a condition not previously observed in the 20 cases of valvular syphilis found in the literature. Of the 3 cases with microscopic examination of the aortic valve, Jansen's [11-e] showed histological changes, but obviously of a more chronic nature, most like those in the posterior aortic cusp of the present case. The minor changes observed in the anterior mitral leaflet are evidently not inflammatory and possibly represent changes due to the unusual tension caused by the attached subaortic ridge. The etiology of the interstitial and perivascular myocardial fibrosis is not clear. However, in view of the failure to find Aschoff bodies or any other stigmata of rheumatic fever, the demonstration of treponemata in the aortic cusps, valve ring and aorta gives strong presumptive evidence of a syphilitic etiology. The changes are not unlike some of the less extensive cases of syphilitic interstitial myocardial fibrosis described by Warthin.

The clinical signs of aortic stenosis, caused by subaortic stenosis, far overshadowed those of aortic insufficiency (the principal anatomical lesion of the aortic valve), leading to the clinical diagnosis of rheumatic aortic stenosis and insufficiency in a syphilitic patient who did not give a past history of rheumatic infection. The common stenotic lesion of the aortic valve, even at 45 years of age, is rheumatic aortic stenosis, with or without signs of aortic insufficiency, or

of mitral stenosis. In the absence of clubbing of the fingers, which has been observed in less than half of the cases of subaortic stenosis, there was no reason other than evidence of a severe stenotic lesion at the base of the heart over a number of years, without cardiac symptoms, to suspect subaortic stenosis. The combination of syphilitic aortitis and rheumatic disease of the heart, although recognized to be infrequent, undoubtedly occurs more often than is indicated by the few authentic cases reported in the literature.[8] Combined rheumatic and syphilitic disease of the aortic valve has been diagnosed histologically.[8] Nevertheless, when signs of aortic stenosis and insufficiency are present in a syphilitic patient, the usual diagnosis should be rheumatic disease of the aortic valve. In this patient subaortic stenosis introduces a rare cause of the signs of aortic stenosis in syphilitic aortic insufficiency. Other rare causes of signs of a stenotic lesion at the base of the heart in syphilitic aortic insufficiency are: severe fibrocalcific disease of syphilitic aortic cusps, a condition which cannot be differentiated clinically from rheumatic aortic stenosis; a gummatous aneurysm of the upper portion of the interventricular septum projecting into the outflow tracts; rupture of a syphilitic aortic valve with partial orificial obstruction by the detached cusps; and an aneurysm of a sinus of Valsalva.

Injury to the already damaged aortic valve by the active syphilitic process may have been an important factor in the rapid onset of heart failure. If the presence of a congenital anomaly had been suspected a consideration of subacute bacterial endocarditis would have seemed justified. Angina pectoris associated with disease of the aortic valve is not rare; a frequent cause in syphilis is narrowing of the mouth of the coronary arteries. Such a condition in the ostium of one coronary artery was the probable cause of this patient's anginal syndrome.

Summary and Conclusions

The case reported herein is the twenty-first authentic instance of true syphilitic endocarditis of heart valves to be found in the literature. It is the ninth case of such involvement of the aortic valve, the process having extended from the root of the aorta into the posterior cusp. An acute exacerbation in the distal portion of this cusp produced subacute syphilitic endocarditis. Treponemata were pres-

TABLE I. *Cases of Actual Syphilitic (Gummatous or Granulomatous) Invasion of Heart Valves* *

Author	Year	Valve invaded	Origin of syphilitic process	Microscopic examination of invaded valves	Treponema stain
Robinson [11a]	1907	A.V and T.V.	Gumma of I.S.	....	....
Klages [11b]	1912	A.V.	Aneurysm root of aorta and gumma I.S.	....	....
Spalding and VonGlahn [11c]	1921	A.V.	Aorta	....	Present in papillary muscle; valve not examined
Major [11d]	1923	A.V. and P.V.	Gumma of I.S.	....	....
Gallavardin and Josserand [11f]	1927	A.V.	Aorta?	Gummatous endocarditis	....
Jansen [11e] (Case 2)	1927	A.V.	Aneurysm root of aorta	Gummatous endocarditis	....
Norris [11g]	1932	A.V.	Aneurysm of aortic sinus	....	....
Sohval [11o] (Case 1)	1935	A.V.	Gumma of I.S. and root of aorta	Vascularized inflammatory tissue; no giant cells or gummata	....
Author's case	1936	A.V.	Root of aorta	Gummatous endocarditis	Numerous treponemata
Schwalbe [11h]	1890	P.V.	Pulmonary artery	Gummatous endocarditis	....
Stockmann [11i] (Case 1a)	1904	P.V.	Gumma of I.S.	Gummatous endocarditis	....
Holterdorf [11j]	1916	P.V.	Pulmonary artery	....	....
Major [11d] (see above)	1923	P.V. and A.V.	Gumma I.S.	....	....
Kux [11k]	1932	P.V. and T.V.	Pulmonary artery and gumma of I.S.	....	....
Friedman [11e]	1924	M.V.	Aorta?	Large gumma of anterior leaflet	Negative
Staemmler [11m]	1930	M.V.	Aorta	Dense inflammatory tissue with gummata and giant cells	Negative
Sohval [10] (2 cases)	1935	M.V.	Root of aorta	Dense vascularized inflammatory tissue; no giant cells or gummata	Negative
Robinson [11a] (see above)	1907	T.V. and A.V.	Pulmonary artery	....	....
Bridgman and Schmeisser [11n]	1919	T.V.	Gumma of I.S.	....	....
Kux [11k] (see above)	1932	T.V. and A.V.	Pulmonary artery and gumma of I.S.	T.V. gummatous endocarditis	....

	Aortic	Pulmonary	Mitral	Tricuspid
Cases of syphilitic endocarditis	9	5	4	3
Cases with microscopic examination of the valve	4	2	4	1
Cases examined for treponemata in valve	1	0	4	0
Demonstration of treponemata in valve	1	..	0	..
Cases in above table with interference of valvular function by juxtavalvular gummata	2	2	0	0
Cases in literature [11o] with interference of valvular function by juxtaposed gummata without invasion of valves	2	6	0	2
Total cases with interference of valvular function	13	13	4	5

A.V. = Aortic Valve P.V. = Pulmonary Valve I.S. = Interventricular Septum T.V. = Tricuspid Valve M.V. = Mitral Valve

* In discussing Staemmler's case of mitral gummatous endocarditis [11-m] Geipel mentioned an almost identical case, a detailed report of which was not found. Dr. Maude Abbott, in a personal communication, reported the presence of two cases of aortic gummatous endocarditis in the Medical Museum, McGill University. In both cases there is gross evidence of invasion of an aortic cusp from a gumma of the interventricular septum and one case shows a congenitally bicuspid aortic valve. In the earlier literature descriptions of probable cases of gummatous aortic endocarditis were found. (Corrigan, D. J. On permanent patency of the mouth of the aorta, or inadequacy of the aortic valves. *Edinburgh M. & S. J.* 1832, **37**, 225–245. Hope, J. A Treatise on the Diseases of the Heart. Lea and Blanchard, Philadelphia, 1842, Am. Ed. 1.)

ent in the cusp and valve ring. A record of the previous demonstration of treponemata in a cardiac valve was not found.

Congenitally bicuspid aortic valve and subaortic stenosis, two rarely associated cardiac anomalies, were also present. The subaortic stenosis clinically produced signs of aortic stenosis in the anatomical presence of aortic insufficiency, leading to the diagnosis of rheumatic aortic stenosis and insufficiency.

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DESCRIPTION OF PLATE

PLATE 17

FIG. 1. Midportion of the posterior aortic cusp showing a part of the gumma. A = Dense fibrous tissue. B = Area of coagulation necrosis. C = Fibroblastic tissue containing plasma cells, epithelioid, lymphoid and large mononuclear cells. Hematoxylin and eosin stain. × 150.

FIG. 2. An area of the distal portion of the posterior aortic cusp showing numerous treponemata, some of which are in the same focal plane. Warthin-Starry stain. × 2000 (approximately).

Richter

Treponema Pallidum in Syphilitic Valvulitis